IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

MERCK & CO., INC.,	)	
	)	
Plaintiff and Counterclaim Defendant,	)	
	)	
v.	)	C.A. No. 07-229 (GMS)
	)	
RANBAXY INC., and RANBAXY LABORATORIES LIMITED,	)	
	)	
Defendants and Counterclaim Plaintiffs.	)	

MERCK'S MOTION FOR LEAVE TO FILE ITS FIRST SUPPLEMENTAL COMPLAINT

Pursuant to Fed. R. Civ. P. 15(d), plaintiff Merck & Co., Inc. ("Merck") hereby moves for leave to file its First Supplemental Complaint against Ranbaxy Inc. and Ranbaxy Laboratories Limited ("Ranbaxy"). The grounds for this motion are set forth in Merck's Brief In Support Of Its Motion For Leave To File Its First Supplemental Complaint.

Pursuant to D. Del. L.R. 15.1, attached as Exhibit 1 is a copy of Merck's proposed supplemental pleading. Attached as Exhibit A to Exhibit 1 is United States Patent No. 5,147,868 without its certificate of correction, and attached as Exhibit B to Exhibit 1 is United States Patent No. 5,147,868 with its certificate of correction. Attached as Exhibit 2 is a copy of Merck's proposed supplemental pleading indicating the respects in which the supplemental pleading differs from the original pleading.

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Dated: January 11, 2008
1381800

RULE 7.1.1. CERTIFICATION

Counsel for Merck requested that Ranbaxy agree to the filing of Merck's supplemental complaint and Ranbaxy refused on the grounds that it would be futile based on *ISCO International, Inc. v. Conductus, Inc.*, 2002 U.S. Dist. LEXIS 21706 (D. Del. 2002) (Sleet, J.).

Dated: January 11, 2008 */s/ James W. Parrett, Jr. (#4292)*

James W. Parrett, Jr. (#4292)

469779

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

MERCK & CO., INC., Plaintiff and Counterclaim Defendant, v. RANBAXY INC., and RANBAXY LABORATORIES LIMITED, Defendants and Counterclaim Plaintiffs.	)	C.A. No. 07-229 (GMS)

[PROPOSED] ORDER

The Court, having considered plaintiff Merck & Co., Inc.'s Motion for Leave to File Its First Supplemental Complaint;

IT IS ORDERED that Merck's Motion for Leave to File Its First Supplemental Complaint is GRANTED and shall be entered forthwith.

SO ORDERED this ___ day of _____, 2008

_____
United States District Judge

1381807

CERTIFICATE OF SERVICE

I hereby certify that on January 11, 2008, I caused the foregoing to be electronically filed with the Clerk of the Court using CM/ECF which will send electronic notification of such filing to the following:

Frederick L. Cottrell , III, Esquire
RICHARDS, LAYTON & FINGER, P.A.

Kelly E. Farnan, Esquire
RICHARDS, LAYTON & FINGER, P.A.

Additionally, I hereby certify that true and correct copies of the foregoing were caused to be served on January 11, 2008 upon the following individuals in the manner indicated:

BY EMAIL AND HAND DELIVERY

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932280

EXHIBIT 1

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

MERCK & CO., INC., Plaintiff, v. RANBAXY INC., and RANBAXY LABORATORIES LIMITED, Defendants.	)	C.A. No. 07-229 (GMS)

FIRST SUPPLEMENTAL COMPLAINT FOR PATENT INFRINGEMENT

Pursuant to Rule 15(d) of the Federal Rules of Civil Procedure, Plaintiff Merck & Co., Inc. ("Merck"), hereby submits this First Supplemental Complaint for Patent Infringement against defendants, Ranbaxy Inc. and Ranbaxy Laboratories Limited (collectively "Defendants"). Merck alleges as follows:

PARTIES

1. Plaintiff Merck is a corporation incorporated under the laws of New Jersey with its principal place of business at One Merck Drive, Whitehouse Station, New Jersey 08889.

2. On information and belief, defendant Ranbaxy Inc. is a corporation organized and existing under the laws of the state of Delaware, having a principal place of business at 600 College Road East, Princeton, New Jersey 08540. On information and belief, Ranbaxy Inc. is engaged in the development, manufacturing, marketing and sale of pharmaceutical products in the United States, and conducts business in the state of Delaware.

3. On information and belief, defendant Ranbaxy Laboratories Limited ("Ranbaxy Labs") is a corporation organized and existing under the laws of India, having its principal place of business at Plot No. 90, Sector 32, Gurgaon-122 001, Haryana, India. On

information and belief, Ranbaxy Labs is engaged in the development, manufacture, marketing and sale of pharmaceutical products in the United States, and conducts business in the state of Delaware. On information and belief, Ranbaxy Inc. is the wholly owned subsidiary of Ranbaxy Labs and is the agent of Ranbaxy Labs in the United States.

4. On information and belief, the acts of Ranbaxy Inc. asserted herein were done at the direction of, or with the cooperation, participation and assistance of, Ranbaxy Labs.

JURISDICTION AND VENUE

5. This is an action for patent infringement arising under the patent laws of the United States, 35 U.S.C. § 101, *et seq.*, and for declaratory judgment of patent infringement arising under 28 U.S.C. §§ 2201 and 2202 and the patent laws of the United States, 35 U.S.C. § 101, *et seq*. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a) and pursuant to 28 U.S.C. §§ 2201 and 2202.

6. Venue is proper in this Court pursuant to 28 U.S.C. §§ 1391(c) and (d) and 1400(b).

MERCK'S PATENT

7. On September 15, 1992, the United States Patent and Trademark Office ("Patent Office") duly and lawfully issued United States Patent No. 5,147,868 ("the '868 patent," a copy of which is attached as Exhibit A), entitled THIENAMYCIN RENAL PEPTIDASE INHIBITORS, to Merck as assignee of the inventors Donald W. Graham, Edward F. Rogers and Frederick M. Kahan. At all times subsequent to issuance of the '868 patent, Merck has been the owner of the entire right, title and interest in and to the '868 patent, including the right to sue and recover for infringement. The term of the '868 patent expires September 15, 2009. The claims of the '868 patent cover, *inter alia*, the compounds cilastatin and cilastatin sodium.

8. Merck currently sells PRIMAXIN® I.M. which is an injectable suspension containing imipenem and cilastatin sodium. Merck also currently sells PRIMAXIN® I.V., which is an injection containing imipenem and cilastatin sodium.

9. Merck is the holder of approved New Drug Applications ("NDAs") under Section 505 of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 355, for imipenem and cilastatin for injectable suspension (NDA 50-630) and imipenem and cilastatin for injection (NDA 50-587).

DEFENDANTS' ACTIONS

10. Prior to the filing of the original Complaint on April 30, 2007, Defendants filed an Abbreviated New Drug Application ("ANDA") for imipenem-cilastatin and associated drug master file(s), seeking approval to engage in the commercial manufacture, use, and sale of injectable products comprising imipenem and cilastatin sodium ("ANDA products") before the '868 patent expires.

11. By letter dated January 22, 2007, Defendants sent written notice of their filing to Merck, which notice was received by Merck. The notice alleged that Defendants' ANDA products will not infringe any valid claim of the '868 patent. Defendants also informed Merck that Defendants are seeking approval from the FDA to market the ANDA products before the '868 patent expires and that Defendants plan to begin marketing the ANDA products immediately upon approval. Defendants sought a covenant from Merck not to sue under the '868 patent. Merck did not give Defendants a covenant not to sue.

12. On information and belief, Defendants are systematically attempting to meet the applicable regulatory requirements to obtain FDA approval for the ANDA products. On information and belief, Defendants have developed and tested the ANDA products. On

information and belief, Ranbaxy Labs already manufactures and sells a pharmaceutical composition containing cilastatin or cilastatin sodium outside the United States. On information and belief, Defendants are preparing to import the ANDA products into the United States or manufacture the ANDA products in the United States. On information and belief, Defendants have the capacity to begin marketing and manufacturing the ANDA products immediately upon receiving regulatory approval from the FDA.

COUNT I - DECLARATORY JUDGMENT

13. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-12 as though fully set forth herein.

14. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent.

15. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that a suit by Merck will be forthcoming.

16. Defendants' manufacture, use, sale or offer for sale of the ANDA products in the United States or importation of the ANDA products into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

17. An actual controversy now exists between Merck and Defendants with respect to the infringement of the '868 patent.

18. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent.

19. Merck does not have an adequate remedy at law.

20. Merck should be granted relief provided by 35 U.S.C. §283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' ANDA products will infringe the '868 patent and an order of this Court preliminarily and permanently enjoining them from manufacturing, using, selling and offering for sale the ANDA products.

21. On information and belief, Defendants were aware of the existence of the '868 patent and were aware that the marketing, manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the '868 patent.

22. Defendants' written notice of the factual and legal bases for its opinion regarding the alleged invalidity and noninfringement of the '868 patent is devoid of an objective good faith basis in either the facts or the law.

23. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

24. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT II - PATENT INFRINGEMENT

25. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-24 as though fully set forth herein.

26. On information and belief, Defendants filed an ANDA under Section 505(j) of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 355(j), for a drug claimed in the '868 patent.

27. Defendants seek approval of their ANDA to engage in the commercial manufacture, use or sale of a drug or drug formulation claimed in the '868 patent before it expires.

28. Defendants have infringed the '868 patent under 35 U.S.C. § 271(e)(2).

29. Merck should be granted relief provided by 35 U.S.C. § 271(e)(4), including an order of this Court that the effective date of the approval of Ranbaxy's ANDA be a date that is not earlier than the present expiration date of the '868 patent, or any later expiration of exclusivity to which Merck is or becomes entitled and an order of this Court preliminarily and permanently enjoining Defendants from commercially manufacturing, using, selling and offering for sale the ANDA products.

30. On information and belief, Defendants were aware of the existence of the '868 patent and were aware that the manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the '868 patent.

31. Defendants' written notice of the factual and legal bases for its opinion regarding the alleged invalidity and noninfringement of the '868 patent is devoid of an objective good faith basis in either the facts or the law.

32. On information and belief Defendants have acted with willful disregard for Merck's patent rights and have willfully infringed the '868 patent.

33. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT III - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT OF THE '868 PATENT WITH CERTIFICATE OF CORRECTION

34. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-33 as though fully set forth herein.

35. On November 6, 2007, subsequent to the filing of this lawsuit, the Patent Office duly and lawfully issued a Certificate of Correction for the '868 patent under 35 U.S.C. § 255. A copy of the '868 patent with the Certificate of Correction is attached as Exhibit B. The

Certificate of Correction resulted from a Request for Expedited Certificate to Correct the Patent Under 37 CFR 1.323, filed by Merck on May 17, 2007, to correct an error on the face of the '868 patent and an error in the first paragraph of the specification related to the recitation of U.S. Application Serial Number 06/188,178.

36. On November 13, 2007, Merck provided Defendants with a copy of the Certificate of Correction.

37. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent with Certificate of Correction, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

38. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

39. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that the ANDA products will infringe the '868 patent with Certificate of Correction.

40. Defendants' manufacture, use, sale or offer for sale of the ANDA products in the United States or importation of the ANDA products into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

41. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent with Certificate of Correction.

42. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent with Certificate of Correction.

43. Merck does not have an adequate remedy at law.

44. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' ANDA products will infringe the '868 patent with Certificate of Correction and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale the ANDA products.

45. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the '868 patent with Certificate of Correction.

46. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

47. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT IV - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT RELATING TO DEFENDANTS' PRODUCT II

48. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-47 as though fully set forth herein.

49. In summer, 2007, subsequent to the filing of this lawsuit, Defendants filed a second ANDA ("ANDA II") for a different injectable product, which also contains imipenem and cilastatin sodium ("Product II"). Defendants' ANDA II seeks approval to engage in the

commercial manufacture, use, and sale of Product II comprising imipenem and cilastatin sodium before the '868 patent expires.

50. On September 14, 2007, Defendants informed Merck that Ranbaxy had filed their ANDA II. On October 17, 2007, Defendants produced at least a portion of their ANDA II to Merck.

51. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

52. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

53. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that Product II will infringe the '868 patent.

54. Defendants' manufacture, use, sale or offer for sale of Product II in the United States or importation of the Product II into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

55. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent by Product II.

56. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent.

57. Merck does not have an adequate remedy at law.

58. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' Product II will infringe the '868 patent and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale Product II.

59. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of Product II would constitute an act of infringement of the '868 patent

60. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

61. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT V - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT RELATING TO DEFENDANTS' PRODUCT III

62. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-61 as though fully set forth herein.

63. In summer, 2007, subsequent to the filing of this lawsuit, Defendants filed a third ANDA ("ANDA III") for yet another different injectable product, which contains imipenem and cilastatin sodium ("Product III"). Defendants' ANDA III seeks approval to engage in the commercial manufacture, use, and sale of Product III comprising imipenem and cilastatin sodium before the '868 patent expires.

64. On September 14, 2007, Defendants informed Merck that Defendants had filed their ANDA III. On October 17, 2007, Defendants produced at least a portion of their ANDA III to Merck.

65. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

66. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

67. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that Product III will infringe the '868 patent.

68. Defendants' manufacture, use, sale or offer for sale of Product III in the United States or importation of Product III into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

69. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent by Product III.

70. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent.

71. Merck does not have an adequate remedy at law.

72. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' Product III will infringe the '868 patent and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale Product III.

73. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of Product III would constitute an act of infringement of the '868 patent.

74. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

75. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT VI - DECLARATORY JUDGMENT THAT THE '868 PATENT WITH CERTIFICATE OF CORRECTION IS NOT INVALID

76. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-75 as though fully set forth herein.

77. On June 21, 2007, Defendants filed the Answer and Counterclaims of Defendants Ranbaxy Inc. and Ranbaxy Laboratories Limited ("Defendants' Answer and Counterclaims.") Defendants' Answer and Counterclaims asserted, as an affirmative defense and as a counterclaim, that the '868 patent was invalid for "failure to comply with one or more of the requirements of 35 U.S.C. §§ 101, 102, 103, and/or 112."

78. On November 6, 2007, the Patent Office duly and lawfully issued a Certificate of Correction to the '868 patent under 35 U.S.C. § 255.

79. On November 13, 2007, Merck provided Defendants with a copy of the Certificate of Correction.

80. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent with Certificate of Correction, including preparations and activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

81. Defendants have continued to litigate this action and pursue their affirmative defense and counterclaim asserting invalidity of the '868 patent after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

82. The acts of Defendants indicate a refusal to change the course of their actions in spite of the issuance of the Certificate of Correction.

83. An actual controversy now exists between Merck and Defendants with respect to the alleged invalidity of the '868 patent with Certificate of Correction.

84. Merck should be granted relief including an order of this Court declaring that the '868 patent with Certificate of Correction is not invalid.

PRAYER FOR RELIEF

WHEREFORE, plaintiff Merck respectfully requests that:

a. Judgment be entered that Defendants have infringed the '868 patent by submitting the aforesaid ANDA;

b. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' ANDA products;

c. A preliminary and permanent injunction be issued, pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and 28 U.S.C. §§ 2201 and 2202, restraining and enjoining Defendants, their officers, agents, attorneys and employees, and those acting in privity or concert with them, from engaging in the manufacture, use, offer for sale, or sale within the United States, or importation into the United States, of compounds or formulations as claimed in the '868 patent, or from practicing any method as claimed in the '868 patent, or from actively inducing or contributing to infringement of the '868 patent;

d. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA be a date which is not earlier than the present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

e. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' Product II;

f. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA II be a date which is not earlier than the present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

g. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' Product III;

h. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA III be a date which is not earlier than the present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

i. judgment be entered declaring that the '868 patent, as originally issued and with Certificate of Correction, is not invalid;

j. Judgment be entered that Defendants acted with willful disregard for Merck's rights under the '868 patent;

k. Judgment be entered that Defendants have willfully infringed the '868 patent;

l. Judgment be entered that this case is an exceptional one and that Merck should be awarded its reasonable attorneys' fees pursuant to 35 U.S.C. § 285; and

m. Such other and further relief as the Court may deem just and proper under the circumstances.

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Dated: January 11, 2008

EXHIBIT A

US005147868A

United States Patent [19]

Graham et al.

[11] Patent Number: 5,147,868

[45] Date of Patent: Sep. 15, 1992

[54] **THIENAMYCIN RENAL PEPTIDASE INHIBITORS**

[75] Inventors: **Donald W. Graham**, Mountainside; **Edward F. Rogers**, Middletown; **Frederick M. Kahan**, Scotch Plains, all of N.J.

[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

[21] Appl. No.: **839,725**

[22] Filed: **Feb. 19, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 641,317, Jan. 14, 1991, abandoned, which is a continuation of Ser. No. 244,527, Sep. 9, 1988, abandoned, which is a continuation of Ser. No. 878,391, Jun. 19, 1986, abandoned, which is a continuation of Ser. No. 748,300, Jun. 24, 1985, abandoned, which is a continuation of Ser. No. 465,577, Feb. 10, 1983, abandoned, which is a continuation-in-part of Ser. No. 50,233, Jun. 22, 1979, abandoned, which is a continuation-in-part of Ser. No. 927,212, Jul. 24, 1978, abandoned.

[51] **Int. Cl.**[5] **C07C 233/63**; C07C 233/48; C07C 233/47

[52] **U.S. Cl.** **514/119**; 514/547; 514/556; 514/560; 514/563; 558/170; 558/254; 558/442; 560/153; 560/171; 562/15; 562/557; 562/560; 562/561; 562/568; 562/571

[58] **Field of Search** ..... 260/402.3, 403, 404, 260/404.5 R, 401, 402.5; 558/303, 179, 170, 254, 442; 560/147, 149, 155, 169, 172, 153, 171; 562/560, 561, 567, 574, 568, 571; 514/119, 540, 556, 560, 563

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,440,355	4/1948	Behrens	195/96
2,441,130	5/1948	Bernstein et al.	260/402
2,449,191	9/1948	Behrens	260/513
2,460,708	2/1949	Mozingo et al.	260/431
2,483,529	10/1949	Clark	560/39 X
2,569,801	10/1951	Cook et al.	260/307
2,622,074	12/1952	Coover et al.	558/170 X
3,032,581	5/1962	Leonard	560/38
3,949,000	4/1976	Violet	260/606.5 P
3,950,357	4/1976	Kahen et al.	435/119 X
3,960,927	6/1976	Metcalf et al.	260/471 A
3,978,101	8/1976	Violet	260/429 R
4,008,281	2/1977	Knowles et al.	260/606.5 P
4,010,181	3/1977	Violet	260/326.14 T
4,027,037	5/1977	Siegle et al.	424/314

FOREIGN PATENT DOCUMENTS

1354571	5/1974	United Kingdom .

OTHER PUBLICATIONS

Chemical Abstracts 75:76735n (1971).

Richards et al, J. Org. Chem, 41(23), 3674–3677 (1976).

Fleury et al, Bull. de la Soc. Chim. de France, No. 11 1969, 4102–4107.

Srinivasan et al, Tetrahedron Letters, No. 12, pp. 891–894 1976.

Abstract of a Poster Session–*Interscience Conference on Antimicrobial Agents and Chemotherapy*, Sep. 1980.

Srinivasan et al, *Tetrahedron Letters*, No. 12, pp. 891–894 (1976).

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[57] **ABSTRACT**

Novel chemical compounds are provided which selectively inhibit the metabolism of dipeptidase (E.C.3.4.13.11) and therefore are useful in combination with antibacterial products. These chemical compounds are z-2-acylamino-3-monosubstituted propenoates.

24 Claims, No Drawings

THIENAMYCIN RENAL PEPTIDASE INHIBITORS

RELATIONSHIP TO PRIOR APPLICATION

This is a continuation of application Ser. No. 07/641,317, filed Jan. 14, 1991, now abandoned, which was a continuation of application Ser. No. 07/244,527 filed Sep. 9, 1988, now abandoned, which was a continuation of application Ser. No. 06/878,391, filed Jun. 19, 1986, now abandoned, which was a continuation of application Ser. No. 06/748,300, filed Jun. 24, 1985, now abandoned, which was a continuation of application Ser. No. 06/465,577, filed Feb. 10, 1983, now abandoned, which was a continuation-in-part of application Ser. No. 06/050,233, filed Jun. 22, 1979, now abandoned, which was a continuation-in-part of application Ser. No. 05/927,212, filed Jul. 24, 1978, now abandoned.

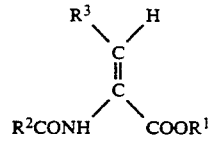
INTRODUCTION

A new class of fused ring β-lactam antibiotics, including thienamycin and its semisynthetic derivatives, epithienamycins, and olivanic acids, has recently been described. These compounds which will be defined more extensively below, are hereinafter referred to as the "thienamycin class of compounds". These compounds have a high level of antibacterial activity, but are subject to extensive metabolism by mammalian species.

The kidney was identified as the primary site of metabolism, and an enzyme was purified from renal extracts which catalyzed the inactivation of thienamycin by hydrolysis of the β-lactam. By such criteria as cytological localization, substrate specificity and susceptibility to enzyme inhibitors, this enzyme is very similar if not identical to a widely studied renal dipeptidase (E.C.3.4.13.11), also referred to in the literature as "dehydropeptidase-I". However, the β-lactamase activity is exhibited only toward the thienamycin class of compounds. Indeed, there exists no precedent example of the mammalian metabolism via β-lactam cleavage of any representative of the classical β-lactam antibiotics, the penicillins and cephalosporins.

DETAILED DESCRIPTION OF THE INVENTION

The chemical substances which selectively inhibit the metabolism of the dipeptidase [E.C.3.4.13.11], also called "dipeptidase inhibitors", include chemical compounds which are Z-2-acylamino-3-monosubstituted propenoates having the following formula



I

wherein R^2 and R^3 are hydrocarbon radicals in the range respectively of 3–10 and 1–15 carbon atoms. In either of these hydrocarbon radicals R^2 and R^3, up to 6 hydrogens may be replaced by halogens, or a non-terminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter.

A terminal hydrogen in R^3 can also be replaced by a hydroxyl or thiol group, which may be acylated, such as with an alkanoyl acid of 1–8 carbon atoms, or carbamoylated, including alkyl and dialkyl carbamate derivatives; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted alkyl amino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as a terminal amino acid grouping.

R^2 is preferably a branched alkyl or cycloalkyl radical (C_{3-10}), with a limitation that the carbon adjacent to the carbonyl cannot be tertiary. R^2 cannot be phenyl or straight chain loweralkyl of 1–4 carbon atoms, where R^3 is straight chain lower alkyl of 1–4 carbon atoms. R^1 is hydrogen, loweralkyl (C_{1-6}) or dialkylaminoalkyl (e.g., $-CH_2CH_2N(C_2H_5)_2$, $-CH_2CH(CH_3)N(CH_3)_2$.

Some of the compounds with formula II above have asymmetric forms. Racemic Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid has been resolved. The activity resides in the dextrorotatory isomer, which has the S-configuration.

Within the definition of R^2, the following sub-groups are included:

$$-R^4 \qquad \text{I A}$$

wherein R^4 is a straight, branched, or cyclic hydrocarbon radical of 3–10 carbon atoms which may be substituted as specified above in the definition of R^2;

$$-R^5R^6 \qquad \text{I B}$$

wherein R^5 is cycloalkyl of 3–6 carbon atoms and R^6 is either 1 or 2 alkyl substituents which may be joined to form another ring on the cycloalkyl group, or R^5 and R^6 may be substituted as specified above in the definition of R^2;

$$-R^7R^8 \qquad \text{I C}$$

wherein R^7 is an alkylene group of 1–3 carbon atoms and R^8 is cycloalkyl of 3–6 carbon atoms which may be substituted as specified above in the definitions of R^2 and R^3;

within these sub-groups, the following specific compounds are included:

I A: Z-2-isovaleramido-2-pentenoic acid; methyl Z-2-isovaleramido-2-butenoate; Z-2-isovaleramido-2-butenoic acid; Z-2-benzamido-2-butenoic acid; Z-2-(3,5,5-trimethylhexanamido)-2-butenoic acid; Z-2-cyclobutanecarboxamido-2-butenoic acid; Z-2-cyclopropanecarboxamido-2-butenoic acid; Z-2-cyclopropanecarboxamido-2-pentenoic acid; Z-2-(3-methylvaleramido)-2-butenoic acid; Z-2-cycloheptanecarboxamido-2-butenoic acid; Z-2-nonanamido-2-butenoic acid; Z-2-cyclohexanecarboxamido-2-butenoic acid; Z-2-(4-methylvaleramido)-2-butenoic acid; Z-2-t-butylacetamido-2-butenoic acid; Z-2-octanamido-2-butenoic acid; Z-2-butyramido-2-butenoic acid; Z-2-valeramido-2-butenoic acid; Z-2-valeramido-2-pentenoic acid; Z-2-cyclopentanecarboxamido-2-butenoic acid; Z-2-(6-methylheptanamido)-2-butenoic acid; Z-2-hexanamido-2-butenoic acid; Z-2-(3,7-dimethyloctanamido)-2-butenoic acid; Z-2-(3,7-dimethyl-6-octenamido)-2-butenoic acid; Z-2-(5-chlorovaleramido)-2-butenoic acid; Z-2-(3-chlorobenzoylamido)-2-butenoic acid; Z-2-(2-chlorobenzamido)-

2-butenoic acid; Z-2-nonanamido-2-butenoic acid; Z-2-(6-bromohexanamido)-2-butenoic acid; Z-2-(3,3-dimethylpropenamido)-2-butenoic acid; Z-2-benzamido-2-cinnamic acid; Z-2-benzamido-2-pentenoic acid; Z-2-benzamido-5-methoxy-2-pentenoic acid; Z-2-benzamido-2-hexenedioic acid; Z-2-isovaleramido-2-octenoic acid; Z-2-isovaleramido-2-cinnamic acid; Z-2-isovaleramido-2-hexenedioic acid; Z-2-cyclopropanecarboxamido-2-cinnamic acid; Z-2-cyclopropanecarboxamido-2-hexenedioic acid; Z-2-(5-methoxy-3methyvaleramido)-2-butenoic acid; Z-2-ethylthioacetamido-2-butenoic acid; Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-ethylhexanamido)-2-butenoic acid; Z-2-di-n-propylacetamido-2-butenoic acid;

B: Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; (+)-Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-cinnamic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methoxy-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4,4,4-trifluoro-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-3-(2-chlorophenyl)propenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenedioic acid; Z-2-(2-ethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-diethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-diethylcyclopropanecarboxamido)- 2-pentenoic acid; Z-2-(2-isopropyl-2-methylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-methylcyclohexanecarboxamido)-2-butenoic acid; Z-5-cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-5-(N,N-dimethylcarbamoyl)-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methanesulfonyl-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-ethoxycarbonyl-2-pentenoic acid; Z-2-(2-methylcyclopropanecarboxamido)-2-butenoic acid; methyl Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoate; ethyl Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoate; 2-dimethylaminoethyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; 3-diethylaminopropyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,3-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(3,3-dimethylcyclobutanecarboxamido)-2-butenoic acid; Z-2-(2-spirocyclopentanecarboxamido)-2-butenoic acid; Z-2-(2-t-butyl-3,3-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4-methyl-2-pentenoic acid; Z-2-(2-t-butylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-phenylcyclopropanecarboxamido)-2-butenoic acid; Z-3-cyclohexyl-2-(2,2-dimethylcyclopropanecarboxamido)-propenoic acid; Z-5-carboxy-5-(2,2-dimethylcyclopropanecarboxamido)-4-pentenamidine; Z-5-dimethyl amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-3-cyclopropyl-2-(2,2-dimethylcyclopropanecarboxamido)propenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2,5-hexadienoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4-phenyl-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-mercapto-2-hexenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methylthio-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-phosphono-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-phenyl-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-tridecenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-methoxy-2-hexenoic acid (and 5-methoxy-2-pentenoic acid); Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-methyl-2 heptenoic acid; Z-4-cyclohexyl-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid;

I C: Z-2-cyclobutylacetamido-2-butenoic acid; Z-2-cyclopentylacetamido-2-butenoic acid; Z-2-cyclohexylacetamido-2-butenoic acid; Z-2-(4-cyclohexylbutyramido)-2-butenoic acid; Z-2-(4-cyclohexylbutyramido)-2-butenoic acid; Z-2-cyclopropylacetamido-2-butenoic acid; Z-2-cyclopropylacetamido-2-pentenoic acid; Z-2-(3-cyclopentylpropionamido)-2-butenoic acid; Z-2-(3-cyclohexylpropionamido)-2-butenoic acid; Z-2-(4-(2-thienyl)-butyramido)-2-butenoic acid; Z-2-(4-phenylbutyramido)-2-butenoic (D,L-α-lipoamido)-2-pentenoic acid; Z-2-(D,L-α-lipoamido)-2-cinnamic acid; Z-2-(3-(2-tetrahydrofuryl)-propionamido)-2-butenoic acid.

Particularly preferred substituents within the definition of R^2 above include the 2,2-dimethylcyclopropyl and the 2,2-dichlorocyclopropyl groups.

Within the definition of R^3, particularly preferred groups of compounds include n-alkyl (1–9 carbons) and n-alkyl (1–9 carbons) having a terminal substituent which is a quaternary nitrogen, amine derivative, or amino acid derived group.

By the term "quaternary nitrogen" is meant a tetrasubstituted or heteroaromatic nitrogen which is positively charged. An ammonium moiety, substituted with hydrocarbon groups having 1–7 carbon atoms, which can be the same or different, is signified.

By the term "amino derivative" is meant a group such as amino, acylamino, ureido, amidino, guanidino and alkyl (1–7 carbon atoms) derivatives thereof.

By the term "amino acid derived group" is meant a moiety such as cysteinyl (—$SCH_2CH(NH_2)COOH$) or sarcosyl (—$N(CH_3)CH_2COOH$) in which a hydrogen joined to O, N or S of known amino acids is replaced.

Particularly preferred compounds from the most preferred groups of substituents of R^2 and R are those wherein R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl, and R^3 is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent, or having a terminal substituent which is trimethylammonium, amidino, guanidino, or 2-amino-2-carboxyethylthio. Names of specific examples of these include:

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid;

Z-8-(L-2 -amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid (racemic and dextrorotatory forms);

Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid;

7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid; and 6-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid.

The Z configuration (J. E. Blackwood et al., *J. Am. Chem. Soc.*, 90, p. 509 (1968)) is assigned to the above compounds on the basis of their NMR spectra by analogy with the work of A. Srinavasan et al. [Tetrahedron Lett., 891 (1976)].

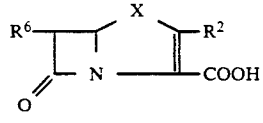
Although these compounds of Formula I, when R^1 is H, are described and named as the free acids, it will be apparent to one skilled in the art that various pharmaceutically acceptable derivatives such as alkali and alkaline earth metal, ammonium, or amine salts, or the like can be employed as equivalents thereto. Salts such as the sodium, potassium, calcium, or tetramethylammonium salts are suitable.

UTILITY OF THE INVENTION

As noted above, the compounds of this invention are dipeptidase (E.C.3.4.13.11) inhibitors, and can be used in combination with antibacterial compounds which are subject to renal degradation. The group of antibiotics of present primary importance for use in combination with the Z-2-acylamino-3-monosubstituted propenoates of this invention are the "thienamycin class of compounds".

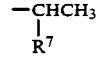
The term "thienamycin class of compounds" is used to identify any of a number of naturally occurring, semi-synthetic, or synthetic derivatives or analog compounds having a common fused-ring β-lactam nucleus. These compounds can be generically classed as 6- and (optionally) 2-substituted pen-2-em-3-carboxylic acids and 1-carbadethia-pen-2-em-3-carboxylic acids or 1-azabicyclo[3.2.0]hept-2-ene-7-one-2-carboxylic acids.

Specific compounds particularly useful in this invention are represented structurally in the following formula II:



II

wherein X can be CH_2 or S; R^2 can be hydrogen; —S—$CH_2CH_2NHR^3$, wherein R^3 is hydrogen, acetyl, formimidoyl, acetimidoyl; —S(O)—CH=CHNH-$COCH_3$ and —S—CH=CHNH$COCH_3$; and R^6 is



wherein R^7 is hydrogen, hydroxy or sulfonyloxy, or R^6 is H. All possible stereoisomeric forms are included within the above structural definition.

All of these compounds within Formula II are described in the literature. When X is CH_2, and R^2 is $SCH_2CH_2NH_2$, and R^6 is $CH(OH)CH_3$, the compound is known as thienamycin, an antibiotic produced by fermentation of *S. cattleya*, described and claimed in U.S. Pat. No. 3,950,357, issued Apr. 13, 1976. The N-substituted derivatives of thienamycin, i.e., in the formula II above wherein R^3 is other than hydrogen, are disclosed and claimed in co-pending U.S. applications and their published foreign equivalents. The fermentation product N-acetyl thienamycin (R^6 is $CH(OH)CH_3$, and R^3 is acetyl), also called 924A, is claimed in Belgian Patent No. 848,346, issued May 16, 1977. The N-imidoyl derivatives are covered in Belgian Patent No. 848,545, issued May 20, 1977. The unsaturated side chain-containing compound, also called N-acetyl-dehydrothienamycin or $924A_5$ is a fermentation product claimed in U.S. Ser. No. 788,491, filed Apr. 18, 1977, Case 16022, now U.S. Pat. No. 4,162,323, issued Jul. 24, 1979, and also Belgian Patent No. 866,035, issued Oct. 17, 1978. Epimeric forms of N-acetyl thienamycin, also called $890A_1$ and $890A_3$, as well as the desacetyl $890A_1$ and desacetyl $890A_3$ are disclosed, respectively in published French Appln. 7,634,887, filed Nov. 19, 1976, with U.S. Ser. No. 634,300, filed U.S. priority of Nov. 21, 1975, case 15745, and Belgian Patent 848,349, issued May 16, 1977. Epimeric forms forms of the unsaturated thienamycin, also called $890A_2$ and $890A_5$ are claimed in published French of Apr. 28, 1976, Case 15839. The 6-sulfonyloxy-containing N-acetyl compounds, also called $890A_9$ or $890A_{10}$, are claimed respectively, in published French Appln. 7,734,456, filed Nov. 16, 1977, with U.S. priority of Nov. 17, 1976, Case 15935, and published French Appln. No. 7,734,457, filed Nov. 16, 1977, U.S. priority of Nov. 17, 1976, Case 15936. Desacetyl analogues of $890A_9$ and $890A_{10}$ are respectively claimed in U.S. Ser. No. 767,723, filed Feb. 11, 1977, Case 15975, now abandoned, and its continuation U.S. Ser. No. 860,665, filed Dec. 15, 1977, now abandoned, and also in French Appln. 7,803,666, filed Feb. 9, 1978; and U.S. Ser. No. 767,920, filed Feb. 11, 1977, Case 15976, now abandoned, and its continuation U.S. Ser. No. 006,959, filed Jan. 25, 1979, now abandoned, and also in French Appln. 7,803,667, filed Feb. 9, 1978. Some of these latter compounds in the $890A_9$ and $890A_{10}$ series are also known as derivatives of olivanic acid (see Corbett et al., *J. Chem. Soc. Chem. Commun.* 1977, No. 24, pp. 953–54). Compounds of the Formula I above when R^2 is hydrogen, also called descysteaminyl thienamycins, are claimed in U.S. Ser. No. 668,898, filed Mar. 22, 1976, Case 15866, now abandoned, and its continuation-in-part, U.S. Ser. No. 847,297, filed Oct. 31, 1977, now abandoned, and also in Belgian Patent 867,227, issued Nov. 20, 1978.

When R^6 is hydrogen, and X is CH_2, these compounds are disclosed in Case 15902, U.S. Ser. No. 843,171, filed Jan. 1, 1977, and in its published German equivalent Off. 2,751,624.1, filed Nov. 18, 1977.

A thienamycin-type antibiotic in which R^2 is —SCH_2CH_2NHAc and R^6 is C_2H_5, has been named PS-5 and is reported by K. Okaimura et al., *J. Antibiotics* 31 p. 480 (1978), see also Belgian Patent 865,578.

The compounds in which X is S, also called "penems", are described by R. B. Woodward in "Recent Advances in the Chemistry of β-Lactam Antibiotics", J. Elks (Ed), The Chemical Society, London, 1977, p. 167; R. B. Woodward, Abstracts of Uppsala University 500 Years Symposium on Current Topics in Drug Research, Uppsala, Sweden, October 1921, 1977. *Acta. Pharm. Suecica*, Vol. 14, Supplement, p. 23, and U.S. Pat. No. 4,070,477, issued Jan. 24, 1978.

Particularly preferred members within the thienamycin class of compounds are the N-formimidoyl and N-acetamidoyl derivatives of thienamycin. The crystalline form of N-formimidoyl thienamycin, which has recently been described, is also useful in the practice of

this invention. An example illustrating a preferred way of making this compound follows:

ILLUSTRATIVE EXAMPLE

N-Formimidoyl thienamycin, (NFT) crystalline

Step A. Benzylformimidate hydrochloride

A 3 l. three-necked flask fitted with an addition funnel, overhead stirrer, and a reflux condenser, was charged with a mixture of benzyl alcohol (125 g., 1.15 mol) formamide (51 g., 1.12 mol) and anhydrous ether (1200 ml.). The mixture was stirred vigorously at room temperature (20°–25° C.) under a nitrogen atmosphere and benzoyl chloride (157 g., 1.12 mol) in 50 ml. of anhydrous ether was added dropwise using the addition funnel. The addition required approximately 50 minutes.

The reaction mixture was stirred an additional 60 minutes at room temperature. The ether was removed by decantation and 300 ml. of acetic anhydride in 500 ml. of anhydrous ether was added. The mixture was stirred 30 minutes at room temperature. The precipitate was allowed to settle and the etheracetic anhydride was again removed by decantation. The solid was collected by filtration, washed with 500 ml. of ether and dried in vacuo over KOH at 25° C. for 2 hrs. to give 130 g. (67%) of benzylformimidate hydrochloride as a white solid.

The product was assayed by NMR δ (DMSO) 5.7 (s, 2H, ϕCH_2), 7.5 (s, 5H, ϕ), 9.0 (s, 1H, HC=N). The product is thermally unstable. It decomposes to formamide and benzyl chloride at 0° C. and above. However, no appreciable decomposition was detected on storage at −20° C. for 2 months.

Step B. Derivatization of Thienamycin

Thienamycin (in the form of a 6 l. aqueous solution, pH=6.5, concentrate from the fermentation broth, containing 28 g. thienamycin) was placed in a large beaker (12 l) and cooled to 0° C. The beaker was equipped with a pH meter and an efficient high speed stirrer. The pH was raised to 8.5 by the careful addition of 3N KOH (KOH was added dropwise via syringe to the stirred solution). The solution was treated with 6 equivalents of solid benzyl formimidate hydrochloride (~100 g.) in portions while maintaining the pH at 8.5+0.3 by the addition of 3N KOH (200 ml.) using a syringe. The addition required 3–5 min. The reaction mixture was stirred for 6 min. at 0° C. and then assayed by liquid chromatography to insure completion of the reaction. The solution was adjusted to pH 7 with 1N HCl. The volume of the reaction mixture was measured, and the solution was assayed by UV. The neutralized reaction mixture was concentrated to 15 g./l. on the reverse osmosis unit at <10° C. The volume of the concentrate was measured and the pH was adjusted to 7.2–7.4, if necessary. The concentrate was filtered through a medium porosity sintered glass funnel to remove any solids present after concentration.

Step C. Dowex 50W×2 Chromatography

The concentrate (750–1000 ml., 15–20 g.) was applied to 0° C. to a precooled 18 l. column of Dowex 50W×2 in the potassium cycle (200–400 mesh resin) and the column was eluted at 0–5° C. with distilled deionized water a flow rate of 90 ml/min. and a head pressure of 0–45 psig.

Forerun fractions of 4 l., 2 l., and one l., were collected followed by 18 fractions of 450 ml. each, and one final fraction of 2 1. Each fraction was assayed by UV (1/100 dilution, NH_2OH extinction was omitted) and the total amount of NFT present in each fraction was calculated. The beginning and end fractions were assayed for liquid chromatography purity and the desired rich cut fractions were combined. The pH of the combined rich cuts was determined by both pH meter and bromothymol blue indicating solutions and was adjusted to pH 7.2–7.4 if necessary. The combined rich cuts (3–4 l.) were then assayed by UV and the total formamidine content was determined, 15–16 g., 75% yield from the column. The rich cuts were concentrated on the reverse osmosis unit at <10° C. as far as possible, then the concentration to 33 g./l. was completed on the circulatory evaporator at less than 28° C. A total volume of about 500 ml. concentrate was obtained.

Step D. Crystallization of N-Formimidoyl Thienamycin

The concentrate from the previous step is adjusted to 7.3, if necessary, and N-formimidoyl thienamycin content assayed by UV, was about 85–90%. The concentrate was filtered through a sintered glass funnel (medium porosity) into a large Erlenmeyer flask. Five volumes (~2200 ml.) of 3A ethanol was filtered into the concentrate and the solution was stirred at room temperature for 10 minutes and at 0° C. for 12–24 hrs.

The crystals were filtered by suction filtration and washed with 0.1 volume (~250 ml.) of 0° C. 80% 3A ethanol followed by 1/25 volume (100 ml.) of 3A ethanol at room temperature. The crystals were dried in vacuo for 12–24 hrs. to give approximately a 40% overall yield of N-formimidoyl thienamycin (10–12 g.).

Analytical results on a 50 g. blend of N-formimidoyl thienamycin, prepared as above, are as follows:

C, theory 45.42%; found, 45.82%

H, theory 6.03%; found, 5.72%

N, theory 13.24%; found, 13.10%

S, theory 10.10%; found, 10.14%

residue on ignition, predicted 0.5, found 0.47%; $[\alpha]_D^{25}=89.4°$, T.G.=6.8%, UV δ max 300 MM, E %=328.

METHODS OF USING THE INVENTION

As mentioned above, the thienamycin-type compound is used in combination with the dipeptidase inhibitor. The combination product is not part of this invention, but is claimed in a copending application, Case 16174, U.S. Ser. No. 927,213, filed Jul. 24, 1978, now abandoned, and in Case 16174IA, U.S. Ser. No. 050,232, filed Jun. 22, 1979, now abandoned, and in Case 16174IB, filed concurrently herewith.

The combination of the novel chemical inhibitors of this invention and the thienamycin class compound can be in the form of a pharmaceutical composition containing the two compounds in a pharmaceutically acceptable carrier. The two can be employed in amounts so that the weight ratio of the thienamycin class compound to inhibitor is 1:3 to 30:1, and preferably 1:1 to 5:1.

The components can also be separately administered. For instance, the thienamycin class compound can be administered intramuscularly or intravenously in amounts of 1–100 mg/kg/day, preferably 1–20 mg/kg/day, or 1–5 mg/kg/day, in divided dosage forms, e.g., three or four times a day. The inhibitor can be separately administered, orally, intramuscularly, or IV, in amounts of 1–100 mg/kg/day, or preferably 1–30 mg/kg/day, or 1–5 mg/kg/day. The amounts of the

two components administered during one day ideally are within the ratio limits denoted above.

One preferred dosage form known to applicants is as a single dose, of two crystalline compounds, one being N-formimidoyl thienamycin and the other being (+) Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, co-administered in a sterile aqueous IV injection form (sodium salt), at a level of 150 mg. of the thienamycin and either 75 or 150 mg of the octenoic acid. This dose is given to humans (each assumed to weigh about 80 kg.) from 1 to 4 times a day, or 2–8 mg/kg/day of the thienamycin class compound and 1–8 mg/kg/day of the inhibitor.

The most preferred dosage regimen and level is the combination of crystalline N-formimidoyl thienamycin and the other being the crystalline form of 7-(L-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid, co-administered in a sterile aqueous IV injection form (sodium salt), at a level of 250 or 500 mg of the thienamycin and about 1:1 (weight) of the heptenoic acid, or 250 or 500 mg. This dose is given to humans (each assumed to weigh about 80 kg.) from 1 to 4 times daily, or 3.1–25 mg/kg/day of each drug.

The components, whether administered separately or together are employed in pharmaceutically acceptable carriers such as conventional vehicles adapted for oral adminstration such as capsules, tablets, or liquid solutions or suspensions. The components separately or together, can also be dissolved in a vehicle adapted for administration by injection. Suitable formulations for oral use, may include diluents, granulating agents, preservatives, binders, flavoring agents, and coating agents. The example of an oral use composition in the combination of active ingredients, or the acid component alone, intermixed in the dry pulverulent state with gelatin, starch, magnesium stearate, and alginic acid, and pressed into a tablet.

As noted above, the presently known preferred method is parenteral administration of the thienamycin class compound and either co-parenteral administration or oral administration of the inhibitor compound.

METHODS OF TESTING THE COMBINATION ANTIBACTERIAL AGENT

As noted, disposition studies with thienamycin, its natural analogs and its semi-synthetic derivatives have revealed a major metabolic degradation pathway of elimination in the various species examined (mouse, rat, dog, chimpanzee, Rhesus monkey). The extent of metabolism is reflected in low urinary recovery and short plasma half-lives. The nature of this degradation was demonstrated to be lactam cleavage by the renal dipeptidase (E.C.3.4.13.11), described first by Bergmann, M. and Schleich, H., *Z. Physiol. Chem.*, 205 65 (1932); see also Greenstein, J. P., *Advances in Enzymology*, Vol. VIII, Wiley-Interscience, (1948), New York, and Campbell, B. J.; Lin, Y-C., Davis, R. V. and Ballew, E., "The Purification and Properties of Particulate Renal Dipeptidase", *Biochim. Biophys. Acta.*, 118, 371 (1966).

In order to demonstrate the ability of the compounds of Formula I to suppress the action of the renal dipeptidase enzyme, an in vitro screen procedure was followed. This measured the ability of compounds to inhibit hydrolysis of glycyldehydrophenylalanine (GDP) by a solubilized preparation of dipeptidase isolated from hog kidneys. The procedure is as follows: to a 1 ml. system containing 50 mM "MOPS" (3-(N-morpholino)-propanesulfonic acid) buffer, pH 7.1, is added 5 μg of lyophilized enzyme, and the test compound at a final concentration of 0.1 mM. After a five minute incubation at 37° C., GDP is added to a final concentration of 0.05 mM. Incubation is continued for 10 minutes, at 37° C. and hydrolysis of GDP is measured by the change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is expressed as the inhibitor binding constant, K_i. This is the concentration of the inhibitor which achieves 50% inhibition of enzyme.

The substrate GDP is employed in preference to thienamycin in this screen because it has a much higher maximal velocity of hydrolysis by renal dipeptidase, thereby reducing the amount of enzyme required. Both GDP and thienamycin have a similar affinity for renal dipeptidase; furthermore, K_i's of inhibitors tested have been identical for the two substrates.

In addition to this in vitro screen procedure, an in vivo screen was followed to measure the test compound's ability to inhibit metabolism as reflected by increase in urinary recovery of thienamycin from the mouse. The procedure involves co-administration of the test compound by the intravenous or subcutaneous route at a dose-rate of 10–100 mg/kg, with 10 mg/kg thienamycin. Thienamycin recovery in the urine over a 4 hour period is then compared with its recovery in a control group to which test compound was not co-administered.

Urinary recovery of thienamycin was measured in all cases with the use of a cylinder or disc diffusion assay, conducted in a manner described in U.S. Pat. No. 3,950,357. This bioassay, with *Staphylococcus aureus* ATCC 6538 as the test organism, has a useful response range from 0.04 μg/ml to 3.0 μg/ml.

Examples which illustrate this invention follow.

SECTION 1. EXAMPLES ILLUSTRATING ACTIVITY

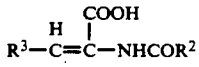
EXAMPLE 1

In Vitro Test Data

A 1 ml. system of 50 mM "MOPS" buffer, pH 7.1, is used. To this is added 5 μg of the pig renal enzyme and an amount of the test compound to bring its final concentration to 0.1 mM. After a five minute incubation at 37° C., an amount of GDP is added to bring its final concentration to 0.05 mM. The system is again incubated for 10 minutes, at 37° C. Hydrolysis of GDP is measured by its change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is presented as percent inhibition. The K_i is a constant indicating the concentration of inhibitor necessary to produce 50% inhibition of enzyme. It is a calculated value obtained from running multiple in vitro assays, as above, at concentrations resulting in inhibition below and above the 50% inhibition point. The results are presented in Table I.

TABLE I

Compounds



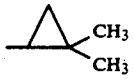
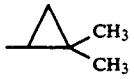
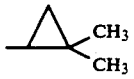
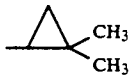
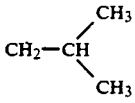
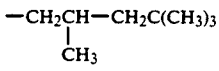
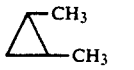
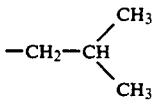
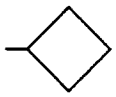
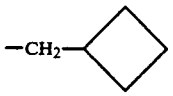
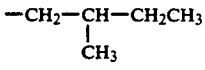
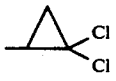
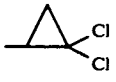
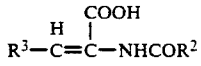
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
1	CH_2CH_3		98	0.18
2*	CH_3		98	0.39
2a*	CH_3		100	0.12
2b*	CH_3			19.8
3	CH_3		92	1.7
4	CH_2CH_3		87	3.2
5	CH_3		81	4.4
6	CH_3		83	4.6
7	CH_3		91	6
8	CH_3		80	6.2
9	CH_3		83	6.6
10	CH_3		97	9
11	CH_3		82	10
12	$-(CH_2)_4CH_2$			0.059
13	$-(CH_2)_5N^+(CH_3)_3$			0.18

TABLE I-continued

Compounds



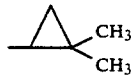
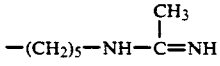
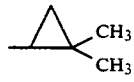
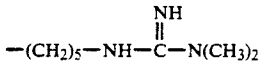
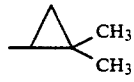
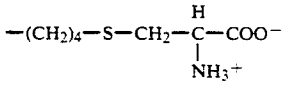
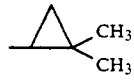
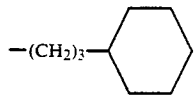
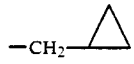
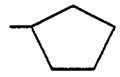
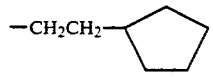
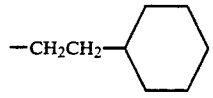
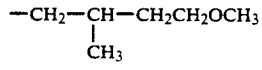
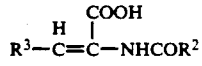
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
14	$-(CH_2)_5N^+(CH_3)_3$			1.11
15				0.72
16				0.89
17				0.21
18	CH_3	$-CH_2C(CH_3)_3$	75	20
19	CH_3	$-(CH_2)_6CH_3$	72	26
20	CH_3	$-(CH_2)_2CH_3$	69	30
21	CH_3		68	30
22	CH_3		64	22
23	CH_3	$(CH_2)_3CH_3$	64	32
24	CH_3		59	30
25	CH_3	$-(CH_2)_4CH(CH_3)_2$	57	
26	CH_3		56	
27	CH_3		54	
28	CH_3	$-CH_2-(CH_2)_3CH_3$	54	39
29	CH_3	$-(CH_2)_5CH_3$	49	
30	CH_3	$-CH(CH_2CH_3)CH_2CH_2CH_2CH_3$	33	
31	CH_3	$-CH(CH_2CH_2CH_3)_2$	13	
32	CH_3	$-CH(CH_3)_2$	31	
33	$HOO-CH_2CH_2$		90	5
34	CH_3		88	9
35	CH_3	$CH_2CH_2CH_2CH_2CH_2Br$	70	19
36	CH_3	$CH_2CH_2CH_2CH_2Cl$	64	20

TABLE I-continued

Compounds



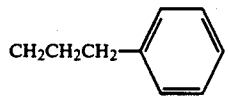
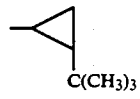
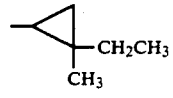
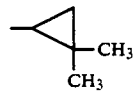
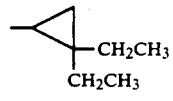
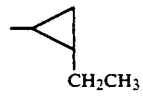
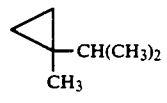
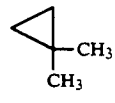
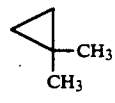
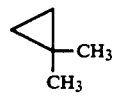
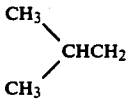
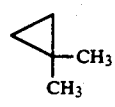
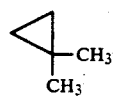
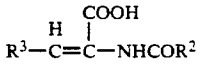
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
37	CH_3		72	11
38	CH_3		90	6.5
39	$CH_3(CH_2)_4$	$CH_2-CH(CH_3)_2$	95	2.6
40	CH_3		100	0.45
41	$(CH_3)_2CH$		98	0.54
42	CH_3		98	0.86
43	CH_3		96	1.6
44	CH_3		95	3
45	CH_3CH_2		98	0.18
46	Ph		100	0.62
47	$CH_3CH_2CH_2$		98	0.11
48			97	0.23
49	$CH_3(CH_2)_3$		100	0.11

TABLE I-continued

Compounds



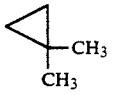
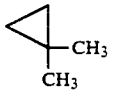
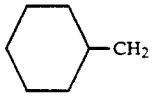
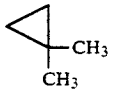
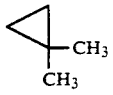
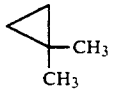
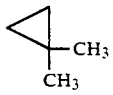
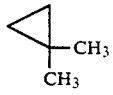
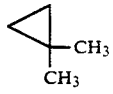
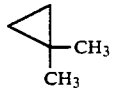
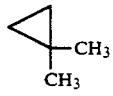
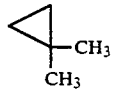
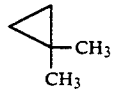
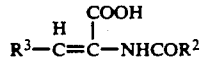
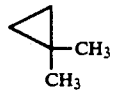
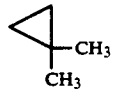
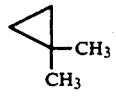
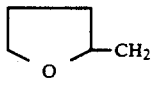
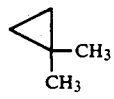
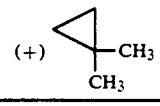
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
50	$CH_3(CH_2)_4$		100	0.17
51	$HOOCCH_2CH_2$		98	0.145
52			100	0.15
53	$PhCH_2CH_2$		96	0.33
54	$CH_3SCH_2CH_2$		99	0.12
55	$CH_3SO_2CH_2CH_2$		96	0.5
56	$CH_3(CH_2)_5$		98	0.149
57	$CH_3(CH_2)_6$		99	0.092
58	$CH_3(CH_2)_9$		96	0.14
59	$PhCH_2$		98	0.44
60	$CH_3O(CH_2)_3$			0.28
61	$CH_3OCH_2CH_2$		98	0.32

TABLE I-continued

Compounds



Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
62	$(CH_3)_3CCH_2$			0.34
63	$(CH_3)_2CHCH_2CH_2$		98	0.15
64	$H_2OC(CH_2)_3$		99	0.048
65				0.39
66	$CH_3(CH_2)_4$			.08

*Compounds 2, 2a, and 2b are the racemic, dextrorotatory and levorotatory forms respectively.

EXAMPLE 2

In Vivo Test Data

An in vivo assay on the mouse was conducted as follows: 20 g Charles River CD, female mice were injected subcutaneously with the chosen dose of the chemical inhibitor. About two minutes later, the dose of thienamycin was given intravenously. A control of thienamycin above was also conducted. The level of thienamycin in the urine as a % of dose was measured using a bioassay technique. Results are found in Table II. The two test compound numbers are those from Table I. Compound 7 is Z-2-isovaleramido-2-butenoic acid; compound 10 is Z-2-cyclopropylcarboxamido-2-butenoic acid.

TABLE II

Compound	Dose, mg/kg Compound	Dose, mg/kg Thienamycin	% Urinary Recovery of Thienamycin
7	50	10	53
7	10	10	53
10	50	10	56
Control	—	10	25–30

EXAMPLE 3

The compounds Z-2-isovaleramido-2-butenoic acid, Compound 7, and Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, compounds were studied, in more detail in vivo in combination with thienamycin (THM), in the mouse. The general test procedure was similar to that of Example 2. Results are summarized in Table III and Table IV.

TABLE III

Effect of Co-administered Z-2-Isovaleramidobutenoic Acid (Compound 7) on the Urinary Recovery of Thienamycin in the Mouse[a]

Route[b]		mg/kg Dose		Urinary Recovery
Compound 7	THM	Compound 7	THM	of THM, %
—	IV or SC	—	10	30 ± 5
SC	SC	0.3	10	33
SC	IV	2	10	42
SC	SC	2	10	47
SC	IV	10	10	53
SC	SC	50	10	54
SC	IV	50	10	53
SC	SC	80	10	59
SC	SC	100	10	81

[a]20 g Charles River, CD_1 female mice
[b]Co-administered

TABLE IV

Effect of Co-administered Z-2-(2,2-Dimethylcyclopropanecarboxamido)-butenoic acid (Compound 2) on Urinary Recovery of Thienamycin in the Mouse[a]

Route[b]		mg/kg Dose		Urinary Recovery
Compound 2	THM	Compound 2	THM	THM, %
—	SC	—	10	30 ± 5
SC	SC	0.1	10	35
SC	SC	0.3	10	40
SC	SC	1	10	46
SC	SC	10	10	60
SC	SC	30	10	73

[a]20 g Charles River, CD_1 female mice
[b]Co-administered

EXAMPLE 4

In another mouse study, the systemic antibacterial activity of thienamycin was enhanced approximately three-fold by coadministering Z-2-isovaleramido-2-butenoic acid, see Table V.

TABLE V

Effect of Co-administered Z-2-Isovaleramido-2-butenoic acid on the Systemic Efficacy of Thienamycin on the Treatment of *Staphalococcus aureus* Infections

		ED_{50}, mg/kg
THM	Alone	0.2
	+100 mg/kg inhibitor	0.06

EXAMPLE 5

A male beagle was used for a study of the effect of dipeptidase inhibitors on the urinary recovery of N-formimidoyl thienamycin. In a control study, the dog was given 5 mg/kg IV of the N-formimidoyl thienamycin without inhibitor. A second experiment used the same amount of N-formimidoylthienamycin, but also administered Z-2-isovaleramido-2-butenoic acid in 3 doses, each providing 20 mg/kg of the compound. The first dose was administered just after injection of the N-formimidoylthienamycin, the second at 40 min. and the third at 60 min. The third study employed a single dose (2 mg/kg) of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, administered just before injection of the N-formimidoyl thienamycin. The results are in Table VI.

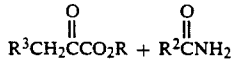
TABLE VI

Urinary Recovery 3 Hours Following the Administration of N-formimidoylthienamycin (5 mg/kg IV) in a Male Beagle

Test Compound	% Urinary Recovery
N-formimidoyl thienamycin	7.8
plus Z-2-isovaleramido-2-butenoic acid	46
plus Z-2-(2,2-dimethylcyclopropane carboxamido)-2-butenoic acid	53

SECTION 2. EXAMPLES ILLUSTRATING CHEMICAL PREPARATIONS

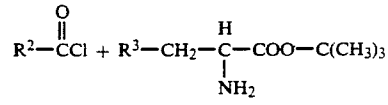
The inhibitor compounds are made by condensing directly the appropriate 2-keto acid or ester and an amide:



III IV I

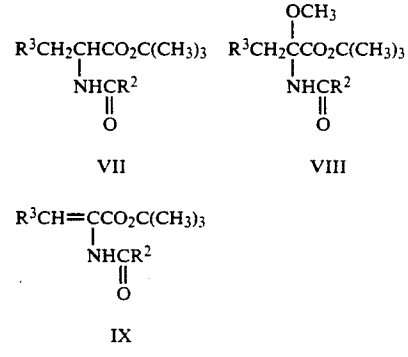
wherein R^2 and R^3 are as defined, and R is hydrogen or alkyl. The general reaction conditions involve mixing approximately 1–4:1 parts of the acid to the amide in an inert solvent such as toluene or methyl isovalerate and heating at reflux with azeotropic removal of water for from 3–48 hours, preferably 5–24 hours. The solution when cooled normally yields the product in crystalline form, but the product can also be isolated using a base extraction process. The product can be recrystallized by using generally known techniques. Condensations of keto esters require use of small amount of p-toluenesulfonic acid as catalyst. The catalyst also is helpful in some condensations with keto acids.

Another route to the novel inhibitor compounds uses an α-amino acid, t-butyl ester in reaction with an acid chloride:



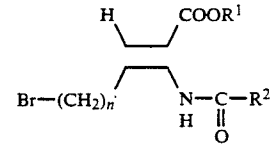
V VI

This reaction takes place in the presence of base, such as triethylamine, in a solvent such as methylene chloride. The resulting N-acylated product (VII) is then oxidized by treatment with t-butyl hypo-chlorite followed by addition of sodium methoxide. This yields the 2-methoxy derivative (VIII) and/or its elimination product, the α,β-unsaturated ester (IX). Further treatment with anhydrous hydrochloric acid converts either VIII or IX (or the mixture of both) to the desired α,β-unsaturated free acid (II).



VII VIII IX

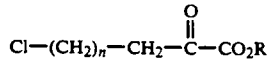
Some compounds wherein R^3 has a terminal substituent which is an amino, quaternary nitrogen, thio derivative, alkoxy, guanidino, acyloxy or cyano can be made most conveniently from an intermediate having a terminal bromine. In this case, the intermediate has the structure



X

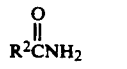
wherein n is the number of carbons in the desired hydrocarbon chain (e.g., from 3–7). In order to prepare R^3 having a terminal trimethylammonium substituent, the bromo intermediate is reacted with trimethylamine; to yield the amino; the bromo intermediate is reacted with ammonia; the guanidino, reaction is with guanidine; to prepare the thio derivatives, including 2-amino-2-carboxyethylthio, the bromo compound is reacted with cysteine HCl, or the appropriate mercaptan. Derivatized amino, such as formamidino, ureido, and acylamido (acetamido) can be made from the compounds having an amino group by reacting with o-benzyl formimidate HCl, potassium cyanate and the appropriate acyl anhydride (acetic dehydride), respectively.

Another route for preparing compounds when R^3 is a terminally substituted thio derivative utilizes a chloro-keto ester intermediate



XI

in reaction with the desired amide,



IV

in toluene at reflux in the presence of a catalytic amount of p-toluene sulfonic acid. The resulting intermediate is hydrolyzed to the acid; the chloro group is then displaced in reaction with the appropriate mercaptan. This reaction is valuable since it permits use of the chiral amide IV, thereby preparing a functionalized side chain. In addition, the mixture of Z+E isomers prepared after the mercaptan condensation can be directly isomerized into the Z form by adding acid to a pH about 3, and heating to about 90° C. for 30 minutes. Only the Z form remains, and recovery is simple and straight forward.

EXAMPLE 6

Z-2-Isovaleramido-2-butenoic Acid

A solution of 1.07 g (10.5 mmole) of 2-ketobutyric acid and 0.71 g (7.0 mmole) of isovaleramide in 15 ml of toluene was stirred under reflux with collection of H_2O in a small Dean-Stark trap. After 5 hrs, the solution was cooled, resulting in fairly heavy crystallization. After standing, the solid was collected on a filter and washed with toluene and then with CH_2Cl_2. Yield of white crystals=0.47 g, mp 172°–174° (slight prelim. softening). The material was recrystallized from diisopropyl ketone. Tlc (4:1 toluene-AcOH) now showed only a faint trace of the other isomer. Yield of white crystals=0.32 g (25%), mp 175° (slight prelim. softening). NMR indicated essentially exclusively Z-isomer.

Anal. ($C_9H_{15}NO_3$)	Calcd.	Found
C	58.36	58.59
H	8.16	8.55
N	7.56	7.43

EXAMPLE 7

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-pentenoic acid

A solution of 1.74 g (15 mmole) of 2-ketovaleric acid and 1.13 g (10 mmole) of 2,2-dimethylcyclopropanecarboxamide in 20 ml of toluene was refluxed with stirring with collection of H_2O in a small Dean-Stark trap. After 20 hrs. the solution was cooled and treated with a gentle stream of N_2. Before much of the solvent had evaporated, crystallization was induced by scratching. After standing, the solid was collected on a filter and washed with toluene and some Et_2O. Yield of white crystals=0.63 g (30%), mp 154.5°–155.5° (slight prelim. softening). Tlc (4:1 toluene-AcOH) showed only an extremely faint trace of the other isomer. NMR was consistent with the Z-configuration.

Anal. ($C_{11}H_{17}NO_3$)	Calcd.	Found
C	62.53	62.86
H	8.11	8.27

-continued

Anal. ($C_{11}H_{17}NO_3$)	Calcd.	Found
N	6.63	6.75

EXAMPLE 8

Z-2-(3-Cyclopentylpropionamido)-2-butenoic acid

A solution of 1.41 g (10 mmole) of 3-cyclopentylpropionamide and 1.53 g (15 mmole) of 2-ketobutyric acid was stirred and refluxed under a small Dean-Stark trap. After 8 hrs. the solution was cooled, resulting in heavy crystallization. The solid was collected on a filter and washed with toluene and CH_2Cl_2. Yield of white crystals=1.44 g, mp 180.5°–182° (prelim. softening). The material was recrystallized from methyl ethyl ketone. Yield of white needles=0.63 g (28%), mp 184°–185° (slight prelim. softening). Tlc (4:1 toluene-AcOH) now showed a single spot, and NMR indicated essentially pure Z-isomer.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.97	63.99
H	8.50	8.67
N	6.22	6.27

EXAMPLE 9

Z-2-(2-Ethylhexanamido)-2-butenoic acid

10 g. of 2-ethylhexanoyl chloride was added dropwise with stirring to 25 ml of cold conc. NH_4OH solution, resulting in immediate precipitation. The mixture was allowed to stir for 2 hrs., then filtered, and air dried to give 6.5 g. of amide. 1.4 g (10 mmole) of the above compound and 1.5 g of ketobutyric acid (15 mmole) were refluxed in 25 ml toluene for 15 hrs with removal of water. The reaction mixture was cooled and partly evaporated with a stream of N_2. Crystallization of product occurred after standing for 3 hrs. The crystals were collected, washed 3× with toluene, and air dried. There was isolated 5 1 13 g (50%) of product, mp 160°–162°. NMR was in accord with the assigned structure and indicated <5% E isomer. Tlc (4:1 toluene-AcOH) showed a single spot.

Anal. ($C_{12}H_{21}NO_3$)	Calcd.	Found
C	63.40	63.63
H	9.30	9.43
N	6.16	5.88

EXAMPLE 10

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-butenoic acid

1.53 g (15 mmoles) of 2-ketobutyric acid, 1.13 g (10 mmoles) of 2,2-dimethylcyclopropanecarboxamide and 20 ml of toluene stirred at reflux for 10 hours. After cooling the crystalline solid was filtered and washed with toluene (3×10 ml) and dried to give 1.06 g of product, mp 140°–141° C. Tlc (4:1 toluene-AcOH) showed essentially one spot and the NMR spectrum fit the desired structure.

Recrystallization from EtOAc gave after drying 0.533 g of product mp 142°–143.5°, homogeneous by tlc.

Anal. ($C_{10}H_{15}NO_3$)	Calcd.	Found
C	60.90	60.92
H	7.67	7.71
N	7.10	7.38

EXAMPLE 11

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenedioic acid

A mixture of 1.0 g. of 2,2-dimethylcyclopropanecarboxamide, 2.4 g. of 2-ketoadipic acid and 25 ml. of methyl isovalerate was heated under reflux for 4 hrs, with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). After standing at room temperature overnight, the crystalline precipitate was filtered, washed with ether and recrystallized from ethyl acetate to give 0.23 g. of product, m.p. 163°–165°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{17}NO_5$)	Calcd.	Found
C	56.46	56.20
H	6.71	6.83
N	5.49	5.32

EXAMPLE 12

Z-2-(2,2-Diethylcyclopropanecarboxamido)-2-butenoic acid

A mixture of 2.3 g of 2-ketobutyric acid, 2.0 g of 2,2-diethylcyclopropanecarboxamide, and 25 ml of toluene was heated under reflux for 16 hrs with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). No product precipitated upon cooling. Ether (25 ml) was added and the mixture was extracted with saturated $NaHCO_3$ (3 times). The combined extracts were acidified with concentrated HCl. The gummy precipitate crystallized when triturated with water. Recrystallization from ethyl acetate gave 0.31 g of product, m.p. 129°–30°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.98	64.01
H	8.50	8.62
N	6.22	6.21

EXAMPLE 13

2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenoic acid

Step A: DL-Norleucine t-butyl ester

General procedure of R. Roeske, *J. Org. Chem.* 28, 1251 (1963).

To a suspension of 9.82 g (75 mmole) of DL-norleucine in 80 ml of dioxane in a 500 ml. pressure bottle cooled in an ice bath was added slowly (with swirling) 8 ml of concentrated H_2SO_4. The resulting mixture was cooled in a dry ice bath as 80 ml of liquid isobutylene was added. The mixture was allowed to warm to room temperature and shaken under autogenous pressure for ~23 hrs. After most of the isobutylene had been vented off, the slightly hazy solution was cooled in ice and then added to a cold mixture of 400 ml of 1N NaOH and 500 ml of Et_2O. After shaking in a separate funnel, the layers were separated, and the aqueous fraction was washed with an additional 100 ml of Et_2O. The Et_2O solution was shaken with 150 ml of 0.5 N HCl. The acidic aqueous fraction was treated with 2.5 N NaOH until strongly basic and then shaken with 250 ml. of Et_2O. The Et_2O solution was dried ($MgSO_4$), filtered, and concentrated on the rotovac. After prolonged pumping on high vacuum over a steam bath, final yield of clear, colorless residual oil = 9.04 g (65%). NMR now showed only a trace of dioxane. TLC (9:1 $CHCl_3$-MeOH) showed a single spot.

Step B N-(2,2-Dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester

To a solution of 8.98 g (48 mmole) of DL-norleucine t-butyl ester and 5.05 g (50 mmole) of triethylamine in 100 ml of CH_2Cl_2 stirred in an ice bath under a drying tube was added dropwise (over a period of 75 min.) a solution of 6.39 g (48 mmole) of 2,2-dimethylcyclopropanecarbonyl chloride (M. Elliot and N. R. James, British Patent No. 1,260,847 (1972)) in 50 ml of CH_2Cl_2. Precipitation of Et_3N HCl occurred during the addition, especially toward the end. As the ice gradually melted, the mixture was allowed to warm to room temperature. After 16 hrs, the mixture was shaken with 200 ml of 0.5 N HCl. The CH_2Cl_2 fraction was washed with an additional 200 ml of 0.5N HCl, then with 2×200 ml of 0.5 N NaOH, and finally 200 ml of H_2O. The CH_2Cl_2 fraction was dried with $MgSO_4$, treated with charcoal, and filtered through Celite. The filtrate was concentrated on the rotovac (finally under high vacuum). Yield of light orange residual oil = 11.93 g (88%). Tlc (2:1 hexane-EtOAc) showed a single spot. NMR and IR were in accord with the assigned structure. After standing for several days, the unused porition of this material crystallized: m.p. 52°–>65°.

Step C: t-Butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate

Based on procedure of H. Poisel and V. Schmidt, *Chem. Ber.*, 108 2547 (1975).

To a solution of 6.37 g (22.5 mmole) of N-(2,2-dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester in 35 ml of Et_2O stirred at room temperature under N_2 in the dark was added 2.69 ml (2.45 g, 22.5 mmole) of t-butyl hypochlorite. After 15 min., a solution of sodium methoxide prepared by dissolving 0.52 g (22.6 mmole) of sodium in 35 ml of MeOH was added. Stirring was continued at ambient temperature under N_2 in the dark. After 16.5 hrs., the precipitated NaCl was filtered off. The filtrate was diluted with Et_2O and washed successively with 3×50 ml of 0.5 N HCl, 50 ml of saturated Na_2CO_3, and 2×50 ml of H_2O. The Et_2O phase was dried over $MgSO_4$ and filtered. The filtrate was concentrated on the rotovac. The pale, golden-yellow residual oil (6.45 g) was subjected to preparative high pressure liquid chromatography, resulting in the separation and isolation of 273 mg and 496 mg of the two diastereomers of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate (respective mp's 114°–118° and 124°–125.5°) as well as 1.97 g of a single isomer (apparently Z) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate (color-less oil).

Step D: 2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenoic acid

A solution of 0.84 g (3.0 mmole) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate in 10

ml of Et_2O saturated with anhydrous HCl was allowed to stand at room temperature under a drying tube. After 17 hrs, the solution was evaporated, and the residual gum was dissolved in 10 ml of saturated $NaHCO_3$. This solution was washed with an additional 15 ml of 0.5 N HCl, then dried ($MgSO_4$), filtered, and concentrated to give a viscous oil. The oil was crystallized from toluene. Yield of white crystals =0.32 g (47%), m.p. 119°–122°. TLC (4:1 toluene-AcOH) showed a single spot. NMR indicated essentially pure Z-isomer. (Note: Treatment of the methanol adduct, t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexenoate, with anhydrous HCl in Et_2O under similar conditions gave the same product.)

EXAMPLE 14

(+)-Z-2-(2,2-Dimethylcyclopropanecarbonylamino)-2-octenoic acid, sodium salt

The reagents, (+)-2,2-dimethylcyclopropanecarboxamide, 7.0 g.; 2-keto-octanoic acid ethyl ester, 14.7 g.; 50 mg. of p-toluene sulfonic acid; and 100 ml. of toluene was changed to a 250 ml. three-necked flask under a Dean Stark trap containing several molecular sieve pellets. The mixture was refluxed vigorously for 27 hours. The resultant light yellow solution was cooled and concentrated in vacuo, at a water bath temperature of 45° C., in the presence of water to help remove toluene. The gummy residue was suspended in 230 ml. of 2N NaOH and stirred at 30° C. for 3 hours; then the temperature was raised to 35° C. for an additional 2½ hrs. until a clear solution formed. The solution was then cooled, 85 ml. methylene chloride added, and the pH adjusted to 8.5 using 4N HCl with stirring. The organic layer was separated and discarded. The aqueous layer (366 ml.) was assayed by liquid chromatography to contain 37.2 mg/ml; 87% Z isomer. Another 85 ml. portion of CH_2Cl_2 was then added and pH adjusted to 4.5 with stirring. The organic layer was separated and the aqueous layer reextracted with 50 ml. of CH_2Cl_2, with the pH again adjusted to 4.5. Combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to a gum. This residue was dissolved in 150 ml. isopropanol and 15 ml. water and the pH adjusted to 8.2 with 2N NaOH. The resulting solution was concentrated to an oily residue which was flushed with isopropanol until it turned to a crystalline solid, indicating that most water had been removed. It was crystallized from 120 ml. of isopropanol, (cooled in ice for 1 hour) filtered, and washed with 50 ml. cold isopropanol followed by copious amounts of acetone. It was dried at 60° C./0.1 mm/2 hours to yield 10.74 g (63.2%) crystalline material, having essentially a single peak in liquid chromatography, m.p. 241°–243° C.

The starting material, (+)-2,2-dimethylcyclopropanecarboxamide is most conveniently prepared by resolution of the D,L acid, followed by reaction with oxalyl chloride and then ammonia to give the resolved amide.

One way of making the starting material is as follows: 23.1 g. of D,L-2,2-dimethylcyclopropanecarboxylic acid was suspended in 33 ml H_2O and the pH adjusted to 8.0, using 50% NaOH, about 10 ml. To this was added a solution of 38.4 g quinine in a mixture of 60 ml. methanol and 30 ml. H_2O to which had been added about 8 ml of concentrated HCl in another 30 ml. H_2O to give a pH of 7.1. (This was actually a solution of quinine hydrochloride.)

These solutions were added all at once, with stirring. The gummy crystalline material which formed was heated to give two clear layers and again stirred vigorously while cooling to give a crystalline product. This product was permitted to stand over two days at room temperature. It was then filtered, washed with 2×10 ml water, and 2×10 ml 50% methanol, and air dried with suction. The yield of crude quinine salt was 44.8 g (48.7% yield) monohydrate, m.p. 113°–116° C., having a $[\alpha]_D^{20}$ of −94.3°, C=1.0; $CHCl_3$. This material was recrystallized from acetone to yield 24.35 g, m.p. 127°–130° C. This purified quinine salt was converted to the acid by reaction with aqueous base and chloroform, followed by acid, to yield (96%) 3.9 g having $[\alpha]_D^{20}$ of +146.0°.

This acid was converted to the amide as follows: A charge of 30.5 g (+)acid was added over 5–10 minutes through a dropping funnel to chilled (10° C.) oxalyl chloride, 54 ml., containing 1 drop dimethylformamide. This was stirred overnight at ambient temperature. A clear solution was observed, which was added to 100 ml. methylene chloride to dilute. Excess oxalyl chloride was removed by concentrating and the mixture flushed twice with methylene chloride.

The resultant solution was diluted with an equal volume of methylene chloride, and added continuously through a dropping funnel to about 100 ml. anhydrous liquid ammonia which was diluted with 100 ml methylene chloride. A dry ice-acetone cooling bath was used during the addition. When all was added, the cooling bath was removed and the mixture stirred at room temperature for about ½ hour. The mixture was filtered, to remove precipitated ammonium chloride, and concentrated to dryness. The crude weight was 26.6 g. (88%). excess hot ethyl acetate and filtered through a preheated sintered glass funnel to separate from trace NH_4Cl. Excess ethyl acetate was atmospherically distilled off. When half the volume remained, 130 ml of heptane were added, and ethyl acetate was continued to be distilled off, until the boiling point started to rise (to near 80° C.; much of product had already crystallized out). Heat was removed, and the mixture let cool gradually to about 30° C., then cooled with an ice bath to 0°–5° C. for about ½ hour. The product was recovered as nice silvery-white crystalline flakes, washed with 3× ethyl acetate/hexane mixture, 1/1.5 and air dried to constant weight. It weighed 23.3 g (77.1% yield overall, 87.6% recovery from crude), m.p.=135°–138° C. (varies with rate of heating). Angle of rotation was determined by dissolving 0.0543 g in 10 ml chloroform, $[\alpha]_D^{20}$ = +100.9°.

EXAMPLE 15

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

Step A: 2,2-Dichlorocyclopropanecarboxamide

A 7.1 g sample of 2,2-dichlorocyclopropanecarbonyl chloride (U.S. Pat. No. 3,301,896, issued Jan. 31, 1967) was added dropwise to 75 ml of concentrated ammonium hydroxide with vigorous stirring. The temperature of the reaction mixture was maintained below 10° C. with an ice bath. The mixture was stirred in the ice bath for 30 min., then at room temperature for 1 hr. The aqueous ammonia was evaporated under reduced pressure (bath at 50° C.). The solid residue was extracted with hot ethyl acetate (3×30 ml). The extracts were boiled down to 40 ml and 20 ml of hexane was added.

After cooling in ice, the solid was filtered, washed with ethyl acetate-hexane (1:1) and dried to give 2.7 g of 2,2-dichlorocyclopropanecarboxamide, m.p. 144–146°. The NMR spectrum was in accord with the desired structure.

Anal. ($C_4H_5Cl_2NO$)	Calcd.	Found
C	31.20	31.26
H	3.27	3.31
N	9.10	9.11
Cl	46.04	45.79

Another 1.3 g of amide, m.p. 143°–145° could be recovered from the mother liquor.

Step B: Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

A mixture of 1.53 g (15 mmoles) of 2-ketobutyric acid, 1.54 g (10 mmoles) of 2,2-dichlorocyclopropanecarboxamide and 10 ml of toluene was heated under reflux for 12 hrs. with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). An additional 0.7 g of 2-ketobutyric acid was added and the reaction mixture was heated under reflux for an additional 12 hrs. The mixture was cooled, diluted with 20 ml of toluene and extracted with saturated sodium bicarbonate (3×10 ml). The extracts were combined, washed with ether and acidified to pH 3 (pH meter) with concentrated hydrochloric acid. A gum precipitated which soon solidified. It was filtered, washed with water, dried and recrystallized from nitromethane to give 423 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-butenoic acid, m.p. 188°–189.5° C. The NMR spectrum was in accord with the desired structure.

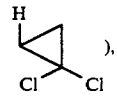
Anal. ($C_8H_9Cl_2NO_3$)	Calcd.	Found
C	40.36	40.48
H	3.81	3.80
N	5.88	5.91
Cl	29.78	29.53

EXAMPLE 16

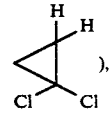
Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-octenoic acid

A mixture of 1.19 g (7.5 mmoles) of 2-ketooctanoic acid, 0.77 g (5.0 mmoles) of 2,2-dichlorocyclopropanecarboxamide, and 5 ml toluene were reacted using the same procedure as in the previous example. The crude product (537 mg) was purified by conversion to the methyl ester (BF_3/CH_3OH), preparative TLC (silica gel G, 4:1 hexane-EtOAc) and saponification of the pure Z-methyl ester (0.3M LiOH/CH_3OH) to give 88 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid as a partially crystalline gum. NMR spectrum (DMSO-d_6): δ9.68 (s, 1H, NH), 6.50 δ (t, 1H,

2.83δ (t, 1H,



1.97 δ (d, 2H



0.87 δ (t, 3H, CH_3).

EXAMPLE 17

Z-8-Bromo-2-(2,2-Dimethylcyclopropanecarboxamido)-2-octenoic acid

To a suspension of 14.4 g (0.3 mole) of 50% NaH dispersion in 360 ml of toluene cooled in an ice bath and in a N_2 atmosphere was added over 45 min. a solution of 146 g (0.6 moles) of 1,6-dibromohexane and 57.6 g (0.3 mole) of ethyl 1,3-dithiane-2-carboxylate in 120 ml of DMF. The cooling bath was removed and the mixture stirred at room temperature for 20 hrs. The reaction mixture was washed with water (3×210 ml), dried over $MgSO_4$ and evaporated under reduced pressure to give 179.5 g of a yellow oil containing the desired alkylated dithiane, 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

To a suspension of 426 g (2.4 moles) of N-bromosuccinamide in 800 ml of acetonitrile and 200 ml of H_2O was added over 45 min. a solution of the crude dithiane in 100 ml of acetonitrile. The temperature of the reaction mixture was maintained below 25° C. with an ice bath. After stirring at 20° C. for 10 min. the dark red reaction mixture was poured into 2 l. of hexane-CH_2Cl_2 (1:1). The solution was shaken with saturated $NaHSO_3$ (2×400 ml) and water (1×500 ml). Then 400 ml of saturated Na_2CO_3 solution was added in small portions (vigorous CO_2 solution). After the foaming subsided the funnel was shaken and the aqueous phase separated. The organic layer was extracted with saturated Na_2CO_3 solution (400 ml) and water (500 ml) and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave 133.8 g of crude bromo ketoester containing 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

A mixture of 133.8 g of crude bromo ketoester, 133 ml of 50% hydrobromic acid and 267 ml of acetic acid was heated at 90° C. (internal temperature) for 75 min. The dark solution was evaporated under reduced pressure until most of the acetic acid was removed. The residue was dissolved in 500 ml of ether, washed with water (2×100 ml) and extracted with saturated $NaHCO_3$ (3×200 ml). The combined $NaHCO_3$ extracts were extracted with ether (2×100 ml) and acidified with concentrated HCl. The precipitated oil was extracted with ether (3×200 ml). The ether extracts were washed with water (1×100 ml) and saturated brine (1×100 ml) and dried over $MgSO_4$. Removal of the ether under reduced pressure gave 46.2 g of pure bromoketo acid. Homogeneous by TlC (silica gel, 4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired product.

A mixture of 46.1 g (0.194 moles) of the bromoketo acid, 17.6 g (0.156 mole) of 2,2-dimethylcyclopropanecarboxamide and 450 ml of toluene was heated under reflux for 13 hrs., with collection of water in a small Dean-Stark trap. After cooling, the clear reaction mixture was extracted with saturated $NaHCO_3$ solution (4×100 ml). The combined extracts were washed with ether (2×100 ml) and then the pH was adjusted to 3.5 (pH meter) by addition of concentrated HCl. An oil precipitated which soon crystallized. The solid was filtered, washed well with water and dried. Recrystallization from acetonitrile gave 22.5 g of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, m.p. 151°–153° C. Homogeneous by TLC (4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired structure.

Anal. ($C_{14}H_{22}BrNO_3$)	Calcd	Found
C	50.61	50.66
H	6.67	6.96
N	4.22	4.45
Br	24.05	23.95

The following ω-bromo compounds were prepared using the same procedure:

Z-6-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-7-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

Z-9-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid;

Z-10-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid;

Z-8-Bromo-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid.

EXAMPLE 18

Z-8-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid

A solution of 664 mg (2 mmoles) of Z-8-bromo-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 10 ml of 40% aqueous dimethylamine was allowed to stand at room temperature for 4 hrs. The solution was poured onto a 3.5×20 cm column of Dowex 50W-$_x$8 (100–200 mesh, H+-) ion exchange resin and the column eluted with water until the effluent was no longer acidic (~200 ml). The column was then eluted with 300 ml of 2N ammonium hydroxide. The effluent was evaporated under reduced pressure to give 600 mg of a colorless glass. This material was dissolved in 3 ml of ethanol, filtered, and added dropwise to 200 ml of rapidly stirred acetone. A gummy solid precipitated which crystallized upon stirring for two days. The solid was filtered, washed with acetone, and dried to give 445 mg of Z-8-dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid as colorless, hygroscopic crystals, m.p. 101°–112° C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{16}H_{28}N_2O_3.H_2O$)	Calcd.	Found
C	61.12	61.03
H	9.62	9.28
N	8.91	8.67

The following ω-amino derivatives were prepared using essentially the same procedure.

Z-10-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid;

Z-8-Amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-Dimethylamino-2-(2,2-dimethylcylclopropanecarboxamido)-2-heptenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-7-(N-methylpiperazinyl)-2-heptenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-pyrrolidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(N-methylpiperazinyl)-2-octenoic acid;

Z-8-Allylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

(2,2-dimethylcyclopropanecarboxamido)-8-piperidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-propargylamino-2-octenoic acid;

Z-8-N-[1-Deoxy-(1-methylamino)-D-glucityl]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-(1-Adamantylamino)-2-(2,2-dimethylcyclopropanecarboxamido-2-octenoic acid;

Z-8-Diallylamino-2-(2,2-dimethylcyclopropanecarboxamido-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(2-hydroxyethylmethylamino)-2-octenoic acid;

Z-8-[(Carboxylmethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-diethylamino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[tris(-hydroxymethyl)methylamino]-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-10-(N-methylpiperazinyl)-2-decenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)ethylamino-]2-octenoic acid;

EXAMPLE 18 A

Z-8-[(Carboxymethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid

3.32 g. of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, 1.0 g. of $CH_3NHCH_2CO_2H$, 3.5 g. of Na_2Co_3 and 30 ml of water were heated at 80° C. in N_2 for 1.5 hours. After purification, 1.0 g. of product was obtained, calc. for $C_{17}H_{28}N_2O_5.2H_2O$:C, 54,24; H, 8.57; N, 7.44; found: C, 54.40; H,8.34; N, 7.16.

EXAMPLE 18 B

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)ethylamino]-2-octenoic acid

Was prepared by reacting the same bromo intermediate (335.1 mg) with 138.2 mg 1-aminoethane phosphoric acid, and 435 mg Na_2CO_3 in 5 ml water, following essentially the same procedure, Ki=0.16.

EXAMPLE 19

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid

A stream of CH_3SH gas was bubbled through a solution of 162 mg (3 mmoles) of sodium methoxide in 5 ml of methanol for 10 min. with cooling in an ice bath. The

solution was allowed to warm to room temperature and 332 mg (1 mmole) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was added. The solution was heated under reflux for 30 min. in a N_2 atmosphere. Most of the methanol was evaporated under reduced pressure, the residue was dissolved in 10 ml of water and acidified with 2.5 N HCl. The precipitated oil was extracted with ether (3×). The ether extracts were washed with water, saturated brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 178 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid, m.p. 82°–84° C. Homogeneous by TLC (toluene-acetic acid, 4:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}NO_3S$)	Calcd.	Found
C	60.18	60.36
H	8.42	8.68
N	4.68	4.59
S	10.69	10.87

The following compounds were prepared by similar methods.

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ethoxythiocarbonylthio-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(1-methyl-5-tetrazolylthio)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-7-{[(methoxycarbonyl)methyl]thio}-2heptenoic acid;

Z-8-Acetylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-[(2-Amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

6-(L-2-carboxethylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-8-(Carbomethoxymethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-6-(Carbomethoxymethylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-2-(2,2-dimethylcycloproopanecarboxamido)-6-(phosphonomethylthio-2-hexenoic acid.

The compound 7-(L-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid is prepared in a similar fashion as the above example, except that Z-7-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid (prepared as in Example 17) (185 mg, 1.05 mmoles) is dissolved in 2.02 ml NaOH solution (2.0 N), and deoxygenated by bubbling a stream of nitrogen gas through it for a minute. Then cysteine.HCl (185 mg, 1.05 mmoles) is added all at once and the reaction stirred at room temperature in a N_2 atmosphere for 3 hours. The reaction mixture is applied to 2×20 cm column of Dowex 50×4 (100–200 mesh H^+), and eluted with 300 ml H_2O), then 200 ml of 2N NH_3 solution. Ammonia evaporated under reduced pressure to give 284 mg of a yellowish glass. This product is dissolved in 4 ml ethanol, and the insoluble material filtered. The filtrate is added dropwise to rapidly stirred diethylether (150 ml). The solid which precipitates is filtered, washed with ether and dried to yield 171 mg product, having one spot (ninhydrin positive) in TLC (nBuOH, HOAc, H_2O; 4:1:1) rf. about 6; NMR is consistent with the desired structure.

Anal. ($C_{16}H_{26}N_2O_5S$)	Calcd.	Found
C	53.61	52.55
H	7.31	7.40
N	7.81	7.89
S	8.94	9.63

EXAMPLE 19 A

Sodium
Z-7-(L-amino-2-Carboxethylthio)-2-(2,2-dimethyl cyclopropane carboxamido)-2-heptenoic acid

A. Grignard Preparation of Ethyl-7-chloro-2-oxoheptanoate

Equimolar amounts (8 moles each) of 1-bromo-5-chloropentane and magnesium are reacted in tetrahydrofuran (960 ml) at 25° C. The flask is charged with mg. in the THF and the bromochloropentane added over 1 hr, then aged 2 hrs. After the reaction was judged complete, the reaction solution was added (cooled to −15° C.) to 16 moles of diethyloxalate in 1856 ml tetrahydrofuran, while maintaining the temperature at −10° C. 3 N.HCl was added to quench, keeping the temperature below 25° C. After stripping solvents, the calculated yield is 48.8% of the ethyl-1-chloro-6-oxoheptenoate.

B. Condensation and Hydrolysis

S-2,2-dimethylcyclopropyl carboxamide (1017 g), 2143.6 g of ethyl-7-chloro-2-ketoheptanoate, 9 liters of toluene and 12 g of p-toluene sulfonic acid were charged to a 22 L. flask, and heated to reflux with stirring. After 23 hrs., liquid chromatography showed the expected product ratio, and 4 L. of toluene were removed under slightly reduced pressure. The pot was charged with water, neutralized to pH 7 with 2N NaOH, and vacuum distilled leaving a final pot volume of about 5 liters.

This was hydrolyzed by adding 1760 g of 50% aq. NaOH (4 liters water) and stirring overnight. The flask was charged with 4 L. methylene chloride, and pH adjusted to 8.8 using HCl. unreacted amide crystallized out. The organic layers were separated from water, and then evaporated. The gummy residue was dissolved in 8 liters water containing 720 g 50% NaOH, and to this solution was charged 1818 g L. cysteine $HCl.H_2O$, 2 kg ice, 2484 g 50% NaOH and 1 liter water.

The pH of this solution, after aging overnight at room temperature, is adjusted to 3.0 with conc. HCl, and the resulting gummy suspension heated to 95° C. to afford a clear solution. After 30 minutes, no E isomer could be detected by lc. After work-up and purification, the overall yield was 50%. This material was recrystallized from acetonitrile. 1500 g of the recrystallized material was dissolved in 6 liters water and 910 ml 3.88N NaOH, then neutralized to pH 7, and lyophilized to afford 1569 g (98.6%) of the title compound; Analysis: calcd: C, 50.52; H, 6.62; N, 7.36; S, 8.43; Na, 6.04; found: C, 50.71; H, 6.78; N, 7.49; S, 8.52; Na, 5.92.

EXAMPLE 19 B

Z-8-[(2-Amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid was also prepared in a similar manner, to that described in Example 19, above, using 3.3 gm of the bromo intermediate, 1.3 g of $H_2NC(=O)\ CH_2SH$, in 50 ml methanol 1.6 g of product, mp 127°–128° C. was obtained.

EXAMPLE 20

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt

A solution of 996 mg (3 mmoles) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 15 ml of 25% aqueous trimethylamine was allowed to stand at room temperature for 3 hrs. The reaction mixture was poured onto a 2×25 cm column of IRA-410 (50–100 mesh, OH^-) ion exchange resin and eluted with water until the effluent was no longer basic. The effluent was evaporated under reduced pressure to give 800 mg of a colorless glass. This material was dissolved in 20 ml of ethanol, filtered and diluted with 600 ml of acetone. After standing at room temperature overnight the crystalline solid which deposited was filtered, washed with acetone and dried to give 720 mg of Z-2-(2,2-dimethylcyclopropanecarboxamide)-8-trimethylammonium hydroxide-2-octenoic acid inner salt as hygroscopic crystals, m.p. 220°–222° C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{17}H_{30}N_2O_3$)	Calcd	Found
C	65.77	65.78
H	9.74	9.98
N	9.02	8.92

Other quaternary derivatives were prepared using essentially the same procedure; these are

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-pyridinium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(2-hydroxyethyldimethylammonium hydroxide)-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-trimethylammonium hydroxide-2-decenoic acid inner salt;

Z-10-(Benzyldimethylammonium hydroxide)-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid inner salt;

Z-8-(Benzyldimethylammonium hydroxide)-2-(2,2dimethylcyclopropanecarboxamido)-2-decenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-9-trimethylammonium hydroxide-2-nonenoic acid inner salt;

Z-8-(2-Dimethylaminoethyldimethylammonium hydroxide)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid inner salt;

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

EXAMPLE 21

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid

A 350 mg sample of Z-8-amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was dissolved in 10 ml of water and the pH adjusted to 8.5 with 2.5N NaOH. A total of 947 mg of benzyl formimidate hydrochloride was added at room temperature in small portions over 20 min. while the pH was maintained between 8–9 by addition of 2.5N NaOH. After stirring at room temperature for 30 min., the cloudy reaction mixture was extracted with ether (3×) and applied to a 2×2.5 cm column of an AG50W-X4 (Na^+, 200–400 mesh) resin. After elution with water, the fractions containing the product were pooled and evaporated under reduced pressure. This material was dissolved in water and applied to a 2×25 cm column of an AGlX8 (HCO_3^-, 200–400 mesh) resin. After elution with water, the fractions containing pure product were pooled and evaporated under reduced pressure. The residue was dissolved in a few ml of warm ethanol, filtered, and added dropwise to 200 ml of ether with rapid stirring. Filtration and washing with ether gave 243 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid as an amorphous solid. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}N_3O_3 \cdot \frac{1}{2}H_2O$)	Calcd.	Found
C	59.69	60.04
H	8.59	8.64
N	13.92	13.57

The following amidino compounds were prepared using similar procedures:

Z-8-Acetamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-N-Benzylformamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-formamidino-2-decenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(2-imidazolinyl-amino)-2-octenoic acid.

EXAMPLE 22

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid

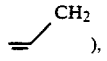
To a solution of 2 mmoles of guanidine (prepared from 432 mg of guanidine sulfate and 630 mg of barium hydroxide octahydrate) in 7 ml of water was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, and the solution was heated at 70° C. in a nitrogen atmosphere for 1 hr. The reaction mixture was applied to a 2×25 cm column of Dowex 50W-X8 (H^+, 100–200 mesh). After elution with water the fractions containing the product were pooled and evaporated under reduced pressure. The residue was dissolved in several ml of warm ethanol and added dropwise to 100 ml of ether with rapid stirring. Filtration and washing with ether gave 107 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid as an amorphous electrostatic powder. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). NMR (D_2O, NaOD): 6.48δ (t, 1H,

);

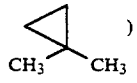
3.10δ (m, 2H,

),

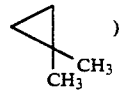
2.10δ (m, 2H,



1.17δ (s, 3H,



1,12 (S, 3H,



The following guanidino compound was prepared using the same procedure:

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(N,N-dimethylguanidino)-2-octenoic acid.

EXAMPLE 23

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic acid

To a solution of 2.43 mmoles of sodium methoxide in 5 ml of methanol was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid. The solution was heated under reflux in a nitrogen atmosphere for 1 hr. The reaction mixture was evaporated under reduced pressure, the residue dissolved in water and acidified with 2.5N hydrochloric acid. The oil which precipitated was extracted with ether (3×). The ether extracts were washed with water, and saturated brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 140 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic acid, m.p. 71°–72° C. Homogeneous by TLC (toluene-HOAc, 4:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}NO_4$)	Calcd.	Found
C	63.58	63.54
H	8.89	9.12
N	4.94	5.16

Using similar procedures, the following compounds were prepared:

Z-8-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

Z-9-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-7-sulfo-2-heptenoic acid sodium salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-sulfo-2-octenoic acid sodium salt;

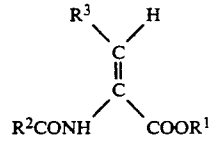
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-hydroxy-2-octenoic acid;

Z-8-Acetoxy-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

The Z-8-cyano-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic compound was prepared from 332 mg 8-bromo-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid and 100 mg NaCN in 2 ml DMSO, heated at 80° C. for 30 min. After extraction and purification, 102 mg of a colorless solid, mp 99°–103° C. were recovered, analysis for $C_{15}H_{22}N_2O_3$: Calcd: C, 64.73; H, 7.97; N, 10.06; Found C, 64.69; H, 8.14; N, 9.41.

What is claimed is:

1. A compound of the formula



R1 is hydrogen or a pharmaceutically acceptable cation;

R2 is X or Y

wherein

X is unsubstituted or substituted branched or linear alkyl of three to ten carbon atoms wherein a non-terminal methylene can be replaced by oxygen, sulfur or SO2, where said substituents are selected from the group consisting of halogen or cycloalkyl of three to six carbon atoms, with the proviso that, when said alkyl is substituted by said cycloalkyl, X is not more than ten total carbon atoms, with the further proviso that not more than six hydrogens of said alkyl can be substituted by said halogen, and with the further proviso that the carbon adjacent to the carbonyl cannot be tertiary;

Y is cycloalkyl of three to six carbon atoms, unsubstituted or substituted with one or two substituents where said substituents are selected from the group consisting of halogen or alkyl of one to four carbon atoms, with the proviso that, when said cycloalkyl is substituted by said alkyl, Y is not more than ten total carbon atoms;

R^3 is unsubstituted or substituted two to fifteen carbon alkyl wherein said substituent is halogen, and wherein a non-terminal methylene can be replaced by oxygen, sulfur or SO_2 and wherein the terminal carbon of said alkyl can be substituted by a moiety selected from the group consisting of amino, ureido, amidino, guanidino, one to four carbon alkylamino, dialkylamino of one to four carbons per alkyl substituent, trialkylammonium, quaternary hydroxyalkyldialkylammonium, acylamino, phosphonylalkylamino, hydroxyalkylamino, formamidino, alkylamidino, N,N-dialkylguanidino, hydroxyl, alkylcarbonyloxy, alkoxycarbonyl, carbamoyl, N,N dialkylcarbamoyl, thiol, acylthio, carboxy, phosphono, cyano, L-2-amino-2-carboxyethylthio or N-methyl-N-carboxymethylamino, with the proviso that no more than six hydrogens of said one to fifteen carbon alkyl can be substituted by halogen, with the further proviso that when R^3 is straight chain lower alkyl of one to four carbon atoms, R^2 cannot be straight chain lower alkyl of one to four carbon atoms, with the further proviso that the compound of the structural formula given above has the Z stereoconfiguration.

2. The compound of claim **1** in which R^2 is 2,2-dimethylcyclopropyl.

3. The compound of claim 1 in which R^2 is 2,2-dichlorocyclopropyl.

4. The compound of claim 1 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)-ethylamino]-2-octenoic acid.

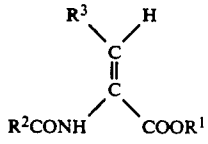
5. The compound of claim 1 which is Z-8-[(carboxymethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

6. The compound of claim 1 which is Z-8-[(2-amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropane-carboxamido)-2-octenoic acid.

7. The compound of claim 1 which is Z-8-cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

8. The compound of claim 1 which is Z-8-acetamido-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

9. A compound of the formula



R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl;

R^1 is hydrogen, loweralkyl of 1–6 carbon atoms, dialkylaminoalkyl, or a pharmaceutically acceptable cation; R^3 is a hydrocarbon chain of 3–7 carbon atoms unsubstituted or substituted with a terminal substituent taken from the group consisting of trimethylammonium, amidino, guanidino, 2-amino-2-carboxyethylthio and ureido.

10. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

11. The compound of claim 9 in which is the 2-dimethylaminoethyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

12. The compound of claim 9 which is Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid.

13. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium-2-octenoic acid inner salt.

14. The compound of claim 9 which is Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium-2-octenoic acid inner salt.

15. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid.

16. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-amidino-2-octenoic acid.

17. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid.

18. The compound of claim 9 which is 6-(L-2-amino-2-carboxyethylthio)-2-(2,2,-dimethylcyclopropanecarboxamido)-2-hexenoic acid.

19. The compound of claim 9 which is 7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid.

20. The compound of claim 19 in the sodium, potassium, calcium or magnesium salt form.

21. The compound of claim 1 in which R^2 is 2,2-dihalocyclopropyl.

22. The compound as claimed in claim 1, in which R^2 is cycloalkyl of three to six carbon atoms substituted by two alkyl substituents of one to three carbon atoms each, witho the proviso that R^2 cannot contain more than ten carbon atoms.

23. A pharmaceutical composition comprising a compound as claimed in claim 1 in an amount sufficient to inhibit the activity of dipeptidase, and a pharmaceutically acceptable carrier.

24. A method of inhibiting the activity of dipeptidase in a mammal in need thereof, comprising the step of administering to said mammal a pharmacologically effective amount of the compound as claimed in claim 1.

* * * * *

EXHIBIT B

US005147868A

United States Patent [19]

Graham et al.

[11] Patent Number: 5,147,868

[45] Date of Patent: Sep. 15, 1992

[54] **THIENAMYCIN RENAL PEPTIDASE INHIBITORS**

[75] Inventors: **Donald W. Graham**, Mountainside; **Edward F. Rogers**, Middletown; **Frederick M. Kahan**, Scotch Plains, all of N.J.

[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

[21] Appl. No.: **839,725**

[22] Filed: **Feb. 19, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 641,317, Jan. 14, 1991, abandoned, which is a continuation of Ser. No. 244,527, Sep. 9, 1988, abandoned, which is a continuation of Ser. No. 878,391, Jun. 19, 1986, abandoned, which is a continuation of Ser. No. 748,300, Jun. 24, 1985, abandoned, which is a continuation of Ser. No. 465,577, Feb. 10, 1983, abandoned, which is a continuation-in-part of Ser. No. 50,233, Jun. 22, 1979, abandoned, which is a continuation-in-part of Ser. No. 927,212, Jul. 24, 1978, abandoned.

[51] **Int. Cl.**[5] **C07C 233/63**; C07C 233/48; C07C 233/47

[52] **U.S. Cl.** **514/119**; 514/547; 514/556; 514/560; 514/563; 558/170; 558/254; 558/442; 560/153; 560/171; 562/15; 562/557; 562/560; 562/561; 562/568; 562/571

[58] **Field of Search** ..... 260/402.3, 403, 404, 260/404.5 R, 401, 402.5; 558/303, 179, 170, 254, 442; 560/147, 149, 155, 169, 172, 153, 171; 562/560, 561, 567, 574, 568, 571; 514/119, 540, 556, 560, 563

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,440,355	4/1948	Behrens	195/96
2,441,130	5/1948	Bernstein et al.	260/402
2,449,191	9/1948	Behrens	260/513
2,460,708	2/1949	Mozingo et al.	260/431
2,483,529	10/1949	Clark	560/39 X
2,569,801	10/1951	Cook et al.	260/307
2,622,074	12/1952	Coover et al.	558/170 X
3,032,581	5/1962	Leonard	560/38
3,949,000	4/1976	Violet	260/606.5 P
3,950,357	4/1976	Kahen et al.	435/119 X
3,960,927	6/1976	Metcalf et al.	260/471 A
3,978,101	8/1976	Violet	260/429 R
4,008,281	2/1977	Knowles et al.	260/606.5 P
4,010,181	3/1977	Violet	260/326.14 T
4,027,037	5/1977	Siegle et al.	424/314

FOREIGN PATENT DOCUMENTS

1354571	5/1974	United Kingdom .

OTHER PUBLICATIONS

Chemical Abstracts 75:76735n (1971).

Richards et al, J. Org. Chem, 41(23), 3674–3677 (1976).

Fleury et al, Bull. de la Soc. Chim. de France, No. 11 1969, 4102–4107.

Srinivasan et al, Tetrahedron Letters, No. 12, pp. 891–894 1976.

Abstract of a Poster Session–*Interscience Conference on Antimicrobial Agents and Chemotherapy*, Sep. 1980.

Srinivasan et al, *Tetrahedron Letters*, No. 12, pp. 891–894 (1976).

Primary Examiner—Carolyn Elmore

Attorney, Agent, or Firm—Frank P. Grassler; Joseph F. DiPrima

[57] **ABSTRACT**

Novel chemical compounds are provided which selectively inhibit the metabolism of dipeptidase (E.C.3.4.13.11) and therefore are useful in combination with antibacterial products. These chemical compounds are z-2-acylamino-3-monosubstituted propenoates.

24 Claims, No Drawings

THIENAMYCIN RENAL PEPTIDASE INHIBITORS

RELATIONSHIP TO PRIOR APPLICATION

This is a continuation of application Ser. No. 07/641,317, filed Jan. 14, 1991, now abandoned, which was a continuation of application Ser. No. 07/244,527 filed Sep. 9, 1988, now abandoned, which was a continuation of application Ser. No. 06/878,391, filed Jun. 19, 1986, now abandoned, which was a continuation of application Ser. No. 06/748,300, filed Jun. 24, 1985, now abandoned, which was a continuation of application Ser. No. 06/465,577, filed Feb. 10, 1983, now abandoned, which was a continuation-in-part of application Ser. No. 06/050,233, filed Jun. 22, 1979, now abandoned, which was a continuation-in-part of application Ser. No. 05/927,212, filed Jul. 24, 1978, now abandoned.

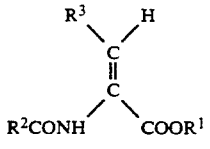
INTRODUCTION

A new class of fused ring β-lactam antibiotics, including thienamycin and its semisynthetic derivatives, epithienamycins, and olivanic acids, has recently been described. These compounds which will be defined more extensively below, are hereinafter referred to as the "thienamycin class of compounds". These compounds have a high level of antibacterial activity, but are subject to extensive metabolism by mammalian species.

The kidney was identified as the primary site of metabolism, and an enzyme was purified from renal extracts which catalyzed the inactivation of thienamycin by hydrolysis of the β-lactam. By such criteria as cytological localization, substrate specificity and susceptibility to enzyme inhibitors, this enzyme is very similar if not identical to a widely studied renal dipeptidase (E.C.3.4.13.11), also referred to in the literature as "dehydropeptidase-I". However, the β-lactamase activity is exhibited only toward the thienamycin class of compounds. Indeed, there exists no precedent example of the mammalian metabolism via β-lactam cleavage of any representative of the classical β-lactam antibiotics, the penicillins and cephalosporins.

DETAILED DESCRIPTION OF THE INVENTION

The chemical substances which selectively inhibit the metabolism of the dipeptidase [E.C.3.4.13.11], also called "dipeptidase inhibitors", include chemical compounds which are Z-2-acylamino-3-monosubstituted propenoates having the following formula



I

wherein R^2 and R^3 are hydrocarbon radicals in the range respectively of 3–10 and 1–15 carbon atoms. In either of these hydrocarbon radicals R^2 and R^3, up to 6 hydrogens may be replaced by halogens, or a non-terminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter.

A terminal hydrogen in R^3 can also be replaced by a hydroxyl or thiol group, which may be acylated, such as with an alkanoyl acid of 1–8 carbon atoms, or carbamoylated, including alkyl and dialkyl carbamate derivatives; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted alkyl amino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as a terminal amino acid grouping.

R^2 is preferably a branched alkyl or cycloalkyl radical (C_{3-10}), with a limitation that the carbon adjacent to the carbonyl cannot be tertiary. R^2 cannot be phenyl or straight chain loweralkyl of 1–4 carbon atoms, where R^3 is straight chain lower alkyl of 1–4 carbon atoms. R^1 is hydrogen, loweralkyl (C_{1-6}) or dialkylaminoalkyl (e.g., $-CH_2CH_2N(C_2H_5)_2$, $-CH_2CH(CH_3)N(CH_3)_2$.

Some of the compounds with formula II above have asymmetric forms. Racemic Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid has been resolved. The activity resides in the dextrorotatory isomer, which has the S-configuration.

Within the definition of R^2, the following sub-groups are included:

$$-R^4 \qquad \text{I A}$$

wherein R^4 is a straight, branched, or cyclic hydrocarbon radical of 3–10 carbon atoms which may be substituted as specified above in the definition of R^2;

$$-R^5R^6 \qquad \text{I B}$$

wherein R^5 is cycloalkyl of 3–6 carbon atoms and R^6 is either 1 or 2 alkyl substituents which may be joined to form another ring on the cycloalkyl group, or R^5 and R^6 may be substituted as specified above in the definition of R^2;

$$-R^7R^8 \qquad \text{I C}$$

wherein R^7 is an alkylene group of 1–3 carbon atoms and R^8 is cycloalkyl of 3–6 carbon atoms which may be substituted as specified above in the definitions of R^2 and R^3;

within these sub-groups, the following specific compounds are included:

I A: Z-2-isovaleramido-2-pentenoic acid; methyl Z-2-isovaleramido-2-butenoate; Z-2-isovaleramido-2-butenoic acid; Z-2-benzamido-2-butenoic acid; Z-2-(3,5,5-trimethylhexanamido)-2-butenoic acid; Z-2-cyclobutanecarboxamido-2-butenoic acid; Z-2-cyclopropanecarboxamido-2-butenoic acid; Z-2-cyclopropanecarboxamido-2-pentenoic acid; Z-2-(3-methylvaleramido)-2-butenoic acid; Z-2-cycloheptanecarboxamido-2-butenoic acid; Z-2-nonanamido-2-butenoic acid; Z-2-cyclohexanecarboxamido-2-butenoic acid; Z-2-(4-methylvaleramido)-2-butenoic acid; Z-2-t-butylacetamido-2-butenoic acid; Z-2-octanamido-2-butenoic acid; Z-2-butyramido-2-butenoic acid; Z-2-valeramido-2-butenoic acid; Z-2-valeramido-2-pentenoic acid; Z-2-cyclopentanecarboxamido-2-butenoic acid; Z-2-(6-methylheptanamido)-2-butenoic acid; Z-2-hexanamido-2-butenoic acid; Z-2-(3,7-dimethyloctanamido)-2-butenoic acid; Z-2-(3,7-dimethyl-6-octenamido)-2-butenoic acid; Z-2-(5-chlorovaleramido)-2-butenoic acid; Z-2-(3-chlorobenzoylamido)-2-butenoic acid; Z-2-(2-chlorobenzamido)-

2-butenoic acid; Z-2-nonanamido-2-butenoic acid; Z-2-(6-bromohexanamido)-2-butenoic acid; Z-2-(3,3-dimethylpropenamido)-2-butenoic acid; Z-2-benzamido-2-cinnamic acid; Z-2-benzamido-2-pentenoic acid; Z-2-benzamido-5-methoxy-2-pentenoic acid; Z-2-benzamido-2-hexenedioic acid; Z-2-isovaleramido-2-octenoic acid; Z-2-isovaleramido-2-cinnamic acid; Z-2-isovaleramido-2-hexenedioic acid; Z-2-cyclopropanecarboxamido-2-cinnamic acid; Z-2-cyclopropanecarboxamido-2-hexenedioic acid; Z-2-(5-methoxy-3methyvaleramido)-2-butenoic acid; Z-2-ethylthioacetamido-2-butenoic acid; Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-ethylhexanamido)-2-butenoic acid; Z-2-di-n-propylacetamido-2-butenoic acid;

B: Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; (+)-Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-cinnamic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methoxy-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4,4,4-trifluoro-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-3-(2-chlorophenyl)propenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenedioic acid; Z-2-(2-ethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-diethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-diethylcyclopropanecarboxamido)- 2-pentenoic acid; Z-2-(2-isopropyl-2-methylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-methylcyclohexanecarboxamido)-2-butenoic acid; Z-5-cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-5-(N,N-dimethylcarbamoyl)-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methanesulfonyl-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-ethoxycarbonyl-2-pentenoic acid; Z-2-(2-methylcyclopropanecarboxamido)-2-butenoic acid; methyl Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoate; ethyl Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoate; 2-dimethylaminoethyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid; 3-diethylaminopropyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-2-(2,3-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(3,3-dimethylcyclobutanecarboxamido)-2-butenoic acid; Z-2-(2-spirocyclopentanecarboxamido)-2-butenoic acid; Z-2-(2-t-butyl-3,3-dimethylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4-methyl-2-pentenoic acid; Z-2-(2-t-butylcyclopropanecarboxamido)-2-butenoic acid; Z-2-(2-phenylcyclopropanecarboxamido)-2-butenoic acid; Z-3-cyclohexyl-2-(2,2-dimethylcyclopropanecarboxamido)-propenoic acid; Z-5-carboxy-5-(2,2-dimethylcyclopropanecarboxamido)-4-pentenamidine; Z-5-dimethyl amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-pentenoic acid; Z-3-cyclopropyl-2-(2,2-dimethylcyclopropanecarboxamido)propenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2,5-hexadienoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-4-phenyl-2-butenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-mercapto-2-hexenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-methylthio-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-phosphono-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-5-phenyl-2-pentenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-tridecenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-methoxy-2-hexenoic acid (and 5-methoxy-2-pentenoic acid); Z-2-(2,2-dimethylcyclopropanecarboxamido)-6-methyl-2 heptenoic acid; Z-4-cyclohexyl-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid;

I C: Z-2-cyclobutylacetamido-2-butenoic acid; Z-2-cyclopentylacetamido-2-butenoic acid; Z-2-cyclohexylacetamido-2-butenoic acid; Z-2-(4-cyclohexylbutyramido)-2-butenoic acid; Z-2-(4-cyclohexylbutyramido)-2-butenoic acid; Z-2-cyclopropylacetamido-2-butenoic acid; Z-2-cyclopropylacetamido-2-pentenoic acid; Z-2-(3-cyclopentylpropionamido)-2-butenoic acid; Z-2-(3-cyclohexylpropionamido)-2-butenoic acid; Z-2-(4-(2-thienyl)-butyramido)-2-butenoic acid; Z-2-(4-phenylbutyramido)-2-butenoic (D,L-α-lipoamido)-2-pentenoic acid; Z-2-(D,L-α-lipoamido)-2-cinnamic acid; Z-2-(3-(2-tetrahydrofuryl)-propionamido)-2-butenoic acid.

Particularly preferred substituents within the definition of R^2 above include the 2,2-dimethylcyclopropyl and the 2,2-dichlorocyclopropyl groups.

Within the definition of R^3, particularly preferred groups of compounds include n-alkyl (1–9 carbons) and n-alkyl (1–9 carbons) having a terminal substituent which is a quaternary nitrogen, amine derivative, or amino acid derived group.

By the term "quaternary nitrogen" is meant a tetrasubstituted or heteroaromatic nitrogen which is positively charged. An ammonium moiety, substituted with hydrocarbon groups having 1–7 carbon atoms, which can be the same or different, is signified.

By the term "amino derivative" is meant a group such as amino, acylamino, ureido, amidino, guanidino and alkyl (1–7 carbon atoms) derivatives thereof.

By the term "amino acid derived group" is meant a moiety such as cysteinyl ($—SCH_2CH(NH_2)COOH$) or sarcosyl ($—N(CH_3)CH_2COOH$) in which a hydrogen joined to O, N or S of known amino acids is replaced.

Particularly preferred compounds from the most preferred groups of substituents of R^2 and R are those wherein R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl, and R^3 is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent, or having a terminal substituent which is trimethylammonium, amidino, guanidino, or 2-amino-2-carboxyethylthio. Names of specific examples of these include:

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid;

Z-8-(L-2 -amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid (racemic and dextrorotatory forms);

Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid;

7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid; and 6-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid.

The Z configuration (J. E. Blackwood et al., *J. Am. Chem. Soc.*, 90, p. 509 (1968)) is assigned to the above compounds on the basis of their NMR spectra by analogy with the work of A. Srinavasan et al. [Tetrahedron Lett., 891 (1976)].

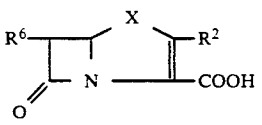
Although these compounds of Formula I, when R^1 is H, are described and named as the free acids, it will be apparent to one skilled in the art that various pharmaceutically acceptable derivatives such as alkali and alkaline earth metal, ammonium, or amine salts, or the like can be employed as equivalents thereto. Salts such as the sodium, potassium, calcium, or tetramethylammonium salts are suitable.

UTILITY OF THE INVENTION

As noted above, the compounds of this invention are dipeptidase (E.C.3.4.13.11) inhibitors, and can be used in combination with antibacterial compounds which are subject to renal degradation. The group of antibiotics of present primary importance for use in combination with the Z-2-acylamino-3-monosubstituted propenoates of this invention are the "thienamycin class of compounds".

The term "thienamycin class of compounds" is used to identify any of a number of naturally occurring, semi-synthetic, or synthetic derivatives or analog compounds having a common fused-ring β-lactam nucleus. These compounds can be generically classed as 6- and (optionally) 2-substituted pen-2-em-3-carboxylic acids and 1-carbadethia-pen-2-em-3-carboxylic acids or 1-azabicyclo[3.2.0]hept-2-ene-7-one-2-carboxylic acids.

Specific compounds particularly useful in this invention are represented structurally in the following formula II:



II

wherein X can be CH_2 or S; R^2 can be hydrogen; —S—$CH_2CH_2NHR^3$, wherein R^3 is hydrogen, acetyl, formimidoyl, acetimidoyl; —S(O)—CH=CHNH-$COCH_3$ and —S—CH=CHNH$COCH_3$; and R^6 is

wherein R^7 is hydrogen, hydroxy or sulfonyloxy, or R^6 is H. All possible stereoisomeric forms are included within the above structural definition.

All of these compounds within Formula II are described in the literature. When X is CH_2, and R^2 is $SCH_2CH_2NH_2$, and R^6 is $CH(OH)CH_3$, the compound is known as thienamycin, an antibiotic produced by fermentation of *S. cattleya*, described and claimed in U.S. Pat. No. 3,950,357, issued Apr. 13, 1976. The N-substituted derivatives of thienamycin, i.e., in the formula II above wherein R^3 is other than hydrogen, are disclosed and claimed in co-pending U.S. applications and their published foreign equivalents. The fermentation product N-acetyl thienamycin (R^6 is $CH(OH)CH_3$, and R^3 is acetyl), also called 924A, is claimed in Belgian Patent No. 848,346, issued May 16, 1977. The N-imidoyl derivatives are covered in Belgian Patent No. 848,545, issued May 20, 1977. The unsaturated side chain-containing compound, also called N-acetyl-dehydrothienamycin or $924A_5$ is a fermentation product claimed in U.S. Ser. No. 788,491, filed Apr. 18, 1977, Case 16022, now U.S. Pat. No. 4,162,323, issued Jul. 24, 1979, and also Belgian Patent No. 866,035, issued Oct. 17, 1978. Epimeric forms of N-acetyl thienamycin, also called $890A_1$ and $890A_3$, as well as the desacetyl $890A_1$ and desacetyl $890A_3$ are disclosed, respectively in published French Appln. 7,634,887, filed Nov. 19, 1976, with U.S. Ser. No. 634,300, filed U.S. priority of Nov. 21, 1975, case 15745, and Belgian Patent 848,349, issued May 16, 1977. Epimeric forms forms of the unsaturated thienamycin, also called $890A_2$ and $890A_5$ are claimed in published French of Apr. 28, 1976, Case 15839. The 6-sulfonyloxy-containing N-acetyl compounds, also called $890A_9$ or $890A_{10}$, are claimed respectively, in published French Appln. 7,734,456, filed Nov. 16, 1977, with U.S. priority of Nov. 17, 1976, Case 15935, and published French Appln. No. 7,734,457, filed Nov. 16, 1977, U.S. priority of Nov. 17, 1976, Case 15936. Desacetyl analogues of $890A_9$ and $890A_{10}$ are respectively claimed in U.S. Ser. No. 767,723, filed Feb. 11, 1977, Case 15975, now abandoned, and its continuation U.S. Ser. No. 860,665, filed Dec. 15, 1977, now abandoned, and also in French Appln. 7,803,666, filed Feb. 9, 1978; and U.S. Ser. No. 767,920, filed Feb. 11, 1977, Case 15976, now abandoned, and its continuation U.S. Ser. No. 006,959, filed Jan. 25, 1979, now abandoned, and also in French Appln. 7,803,667, filed Feb. 9, 1978. Some of these latter compounds in the $890A_9$ and $890A_{10}$ series are also known as derivatives of olivanic acid (see Corbett et al., *J. Chem. Soc. Chem. Commun.* 1977, No. 24, pp. 953–54). Compounds of the Formula I above when R^2 is hydrogen, also called descysteaminyl thienamycins, are claimed in U.S. Ser. No. 668,898, filed Mar. 22, 1976, Case 15866, now abandoned, and its continuation-in-part, U.S. Ser. No. 847,297, filed Oct. 31, 1977, now abandoned, and also in Belgian Patent 867,227, issued Nov. 20, 1978.

When R^6 is hydrogen, and X is CH_2, these compounds are disclosed in Case 15902, U.S. Ser. No. 843,171, filed Jan. 1, 1977, and in its published German equivalent Off. 2,751,624.1, filed Nov. 18, 1977.

A thienamycin-type antibiotic in which R^2 is —SCH_2CH_2NHAc and R^6 is C_2H_5, has been named PS-5 and is reported by K. Okaimura et al., *J. Antibiotics* 31 p. 480 (1978), see also Belgian Patent 865,578.

The compounds in which X is S, also called "penems", are described by R. B. Woodward in "Recent Advances in the Chemistry of β-Lactam Antibiotics", J. Elks (Ed), The Chemical Society, London, 1977, p. 167; R. B. Woodward, Abstracts of Uppsala University 500 Years Symposium on Current Topics in Drug Research, Uppsala, Sweden, October 1921, 1977. *Acta. Pharm. Suecica,* Vol. 14, Supplement, p. 23, and U.S. Pat. No. 4,070,477, issued Jan. 24, 1978.

Particularly preferred members within the thienamycin class of compounds are the N-formimidoyl and N-acetamidoyl derivatives of thienamycin. The crystalline form of N-formimidoyl thienamycin, which has recently been described, is also useful in the practice of

this invention. An example illustrating a preferred way of making this compound follows:

ILLUSTRATIVE EXAMPLE

N-Formimidoyl thienamycin, (NFT) crystalline

Step A. Benzylformimidate hydrochloride

A 3 l. three-necked flask fitted with an addition funnel, overhead stirrer, and a reflux condenser, was charged with a mixture of benzyl alcohol (125 g., 1.15 mol) formamide (51 g., 1.12 mol) and anhydrous ether (1200 ml.). The mixture was stirred vigorously at room temperature (20°–25° C.) under a nitrogen atmosphere and benzoyl chloride (157 g., 1.12 mol) in 50 ml. of anhydrous ether was added dropwise using the addition funnel. The addition required approximately 50 minutes.

The reaction mixture was stirred an additional 60 minutes at room temperature. The ether was removed by decantation and 300 ml. of acetic anhydride in 500 ml. of anhydrous ether was added. The mixture was stirred 30 minutes at room temperature. The precipitate was allowed to settle and the etheracetic anhydride was again removed by decantation. The solid was collected by filtration, washed with 500 ml. of ether and dried in vacuo over KOH at 25° C. for 2 hrs. to give 130 g. (67%) of benzylformimidate hydrochloride as a white solid.

The product was assayed by NMR δ (DMSO) 5.7 (s, 2H, ϕCH_2), 7.5 (s, 5H, ϕ), 9.0 (s, 1H, HC=N). The product is thermally unstable. It decomposes to formamide and benzyl chloride at 0° C. and above. However, no appreciable decomposition was detected on storage at −20° C. for 2 months.

Step B. Derivatization of Thienamycin

Thienamycin (in the form of a 6 l. aqueous solution, pH=6.5, concentrate from the fermentation broth, containing 28 g. thienamycin) was placed in a large beaker (12 l) and cooled to 0° C. The beaker was equipped with a pH meter and an efficient high speed stirrer. The pH was raised to 8.5 by the careful addition of 3N KOH (KOH was added dropwise via syringe to the stirred solution). The solution was treated with 6 equivalents of solid benzyl formimidate hydrochloride (~100 g.) in portions while maintaining the pH at 8.5+0.3 by the addition of 3N KOH (200 ml.) using a syringe. The addition required 3–5 min. The reaction mixture was stirred for 6 min. at 0° C. and then assayed by liquid chromatography to insure completion of the reaction. The solution was adjusted to pH 7 with 1N HCl. The volume of the reaction mixture was measured, and the solution was assayed by UV. The neutralized reaction mixture was concentrated to 15 g./l. on the reverse osmosis unit at <10° C. The volume of the concentrate was measured and the pH was adjusted to 7.2–7.4, if necessary. The concentrate was filtered through a medium porosity sintered glass funnel to remove any solids present after concentration.

Step C. Dowex 50W×2 Chromatography

The concentrate (750–1000 ml., 15–20 g.) was applied to 0° C. to a precooled 18 l. column of Dowex 50W×2 in the potassium cycle (200–400 mesh resin) and the column was eluted at 0–5° C. with distilled deionized water a flow rate of 90 ml/min. and a head pressure of 0–45 psig.

Forerun fractions of 4 l., 2 l., and one l., were collected followed by 18 fractions of 450 ml. each, and one final fraction of 2 l. Each fraction was assayed by UV (1/100 dilution, NH_2OH extinction was omitted) and the total amount of NFT present in each fraction was calculated. The beginning and end fractions were assayed for liquid chromatography purity and the desired rich cut fractions were combined. The pH of the combined rich cuts was determined by both pH meter and bromothymol blue indicating solutions and was adjusted to pH 7.2–7.4 if necessary. The combined rich cuts (3–4 l.) were then assayed by UV and the total formamidine content was determined, 15–16 g., 75% yield from the column. The rich cuts were concentrated on the reverse osmosis unit at <10° C. as far as possible, then the concentration to 33 g./l. was completed on the circulatory evaporator at less than 28° C. A total volume of about 500 ml. concentrate was obtained.

Step D. Crystallization of N-Formimidoyl Thienamycin

The concentrate from the previous step is adjusted to 7.3, if necessary, and N-formimidoyl thienamycin content assayed by UV, was about 85–90%. The concentrate was filtered through a sintered glass funnel (medium porosity) into a large Erlenmeyer flask. Five volumes (~2200 ml.) of 3A ethanol was filtered into the concentrate and the solution was stirred at room temperature for 10 minutes and at 0° C. for 12–24 hrs.

The crystals were filtered by suction filtration and washed with 0.1 volume (~250 ml.) of 0° C. 80% 3A ethanol followed by 1/25 volume (100 ml.) of 3A ethanol at room temperature. The crystals were dried in vacuo for 12–24 hrs. to give approximately a 40% overall yield of N-formimidoyl thienamycin (10–12 g.).

Analytical results on a 50 g. blend of N-formimidoyl thienamycin, prepared as above, are as follows:

C, theory 45.42%; found, 45.82%
H, theory 6.03%; found, 5.72%
N, theory 13.24%; found, 13.10%
S, theory 10.10%; found, 10.14%

residue on ignition, predicted 0.5, found 0.47%; $[\alpha]_D^{25}=89.4°$, T.G.=6.8%, UV δ max 300 MM, E %=328.

METHODS OF USING THE INVENTION

As mentioned above, the thienamycin-type compound is used in combination with the dipeptidase inhibitor. The combination product is not part of this invention, but is claimed in a copending application, Case 16174, U.S. Ser. No. 927,213, filed Jul. 24, 1978, now abandoned, and in Case 16174IA, U.S. Ser. No. 050,232, filed Jun. 22, 1979, now abandoned, and in Case 16174IB, filed concurrently herewith.

The combination of the novel chemical inhibitors of this invention and the thienamycin class compound can be in the form of a pharmaceutical composition containing the two compounds in a pharmaceutically acceptable carrier. The two can be employed in amounts so that the weight ratio of the thienamycin class compound to inhibitor is 1:3 to 30:1, and preferably 1:1 to 5:1.

The components can also be separately administered. For instance, the thienamycin class compound can be administered intramuscularly or intravenously in amounts of 1–100 mg/kg/day, preferably 1–20 mg/kg/day, or 1–5 mg/kg/day, in divided dosage forms, e.g., three or four times a day. The inhibitor can be separately administered, orally, intramuscularly, or IV, in amounts of 1–100 mg/kg/day, or preferably 1–30 mg/kg/day, or 1–5 mg/kg/day. The amounts of the

two components administered during one day ideally are within the ratio limits denoted above.

One preferred dosage form known to applicants is as a single dose, of two crystalline compounds, one being N-formimidoyl thienamycin and the other being (+) Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, co-administered in a sterile aqueous IV injection form (sodium salt), at a level of 150 mg. of the thienamycin and either 75 or 150 mg of the octenoic acid. This dose is given to humans (each assumed to weigh about 80 kg.) from 1 to 4 times a day, or 2–8 mg/kg/day of the thienamycin class compound and 1–8 mg/kg/day of the inhibitor.

The most preferred dosage regimen and level is the combination of crystalline N-formimidoyl thienamycin and the other being the crystalline form of 7-(L-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid, co-administered in a sterile aqueous IV injection form (sodium salt), at a level of 250 or 500 mg of the thienamycin and about 1:1 (weight) of the heptenoic acid, or 250 or 500 mg. This dose is given to humans (each assumed to weigh about 80 kg.) from 1 to 4 times daily, or 3.1–25 mg/kg/day of each drug.

The components, whether administered separately or together are employed in pharmaceutically acceptable carriers such as conventional vehicles adapted for oral adminstration such as capsules, tablets, or liquid solutions or suspensions. The components separately or together, can also be dissolved in a vehicle adapted for administration by injection. Suitable formulations for oral use, may include diluents, granulating agents, preservatives, binders, flavoring agents, and coating agents. The example of an oral use composition in the combination of active ingredients, or the acid component alone, intermixed in the dry pulverulent state with gelatin, starch, magnesium stearate, and alginic acid, and pressed into a tablet.

As noted above, the presently known preferred method is parenteral administration of the thienamycin class compound and either co-parenteral administration or oral administration of the inhibitor compound.

METHODS OF TESTING THE COMBINATION ANTIBACTERIAL AGENT

As noted, disposition studies with thienamycin, its natural analogs and its semi-synthetic derivatives have revealed a major metabolic degradation pathway of elimination in the various species examined (mouse, rat, dog, chimpanzee, Rhesus monkey). The extent of metabolism is reflected in low urinary recovery and short plasma half-lives. The nature of this degradation was demonstrated to be lactam cleavage by the renal dipeptidase (E.C.3.4.13.11), described first by Bergmann, M. and Schleich, H., *Z. Physiol. Chem.*, 205 65 (1932); see also Greenstein, J. P., *Advances in Enzymology*, Vol. VIII, Wiley-Interscience, (1948), New York, and Campbell, B. J.; Lin, Y-C., Davis, R. V. and Ballew, E., "The Purification and Properties of Particulate Renal Dipeptidase", *Biochim. Biophys. Acta.*, 118, 371 (1966).

In order to demonstrate the ability of the compounds of Formula I to suppress the action of the renal dipeptidase enzyme, an in vitro screen procedure was followed. This measured the ability of compounds to inhibit hydrolysis of glycyldehydrophenylalanine (GDP) by a solubilized preparation of dipeptidase isolated from hog kidneys. The procedure is as follows: to a 1 ml. system containing 50 mM "MOPS" (3-(N-morpholino)-propanesulfonic acid) buffer, pH 7.1, is added 5 μg of lyophilized enzyme, and the test compound at a final concentration of 0.1 mM. After a five minute incubation at 37° C., GDP is added to a final concentration of 0.05 mM. Incubation is continued for 10 minutes, at 37° C. and hydrolysis of GDP is measured by the change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is expressed as the inhibitor binding constant, K_i. This is the concentration of the inhibitor which achieves 50% inhibition of enzyme.

The substrate GDP is employed in preference to thienamycin in this screen because it has a much higher maximal velocity of hydrolysis by renal dipeptidase, thereby reducing the amount of enzyme required. Both GDP and thienamycin have a similar affinity for renal dipeptidase; furthermore, K_i's of inhibitors tested have been identical for the two substrates.

In addition to this in vitro screen procedure, an in vivo screen was followed to measure the test compound's ability to inhibit metabolism as reflected by increase in urinary recovery of thienamycin from the mouse. The procedure involves co-administration of the test compound by the intravenous or subcutaneous route at a dose-rate of 10–100 mg/kg, with 10 mg/kg thienamycin. Thienamycin recovery in the urine over a 4 hour period is then compared with its recovery in a control group to which test compound was not co-administered.

Urinary recovery of thienamycin was measured in all cases with the use of a cylinder or disc diffusion assay, conducted in a manner described in U.S. Pat. No. 3,950,357. This bioassay, with *Staphylococcus aureus* ATCC 6538 as the test organism, has a useful response range from 0.04 μg/ml to 3.0 μg/ml.

Examples which illustrate this invention follow.

SECTION 1. EXAMPLES ILLUSTRATING ACTIVITY

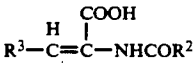
EXAMPLE 1

In Vitro Test Data

A 1 ml. system of 50 mM "MOPS" buffer, pH 7.1, is used. To this is added 5 μg of the pig renal enzyme and an amount of the test compound to bring its final concentration to 0.1 mM. After a five minute incubation at 37° C., an amount of GDP is added to bring its final concentration to 0.05 mM. The system is again incubated for 10 minutes, at 37° C. Hydrolysis of GDP is measured by its change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is presented as percent inhibition. The K_i is a constant indicating the concentration of inhibitor necessary to produce 50% inhibition of enzyme. It is a calculated value obtained from running multiple in vitro assays, as above, at concentrations resulting in inhibition below and above the 50% inhibition point. The results are presented in Table I.

TABLE I

Compounds



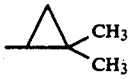
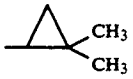
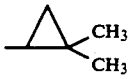
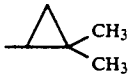
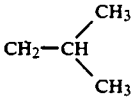
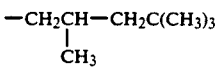
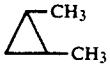
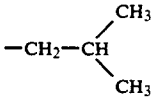
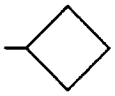
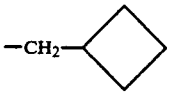
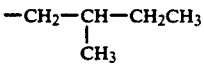
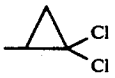
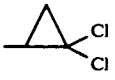
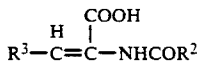
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
1	CH_2CH_3		98	0.18
2*	CH_3		98	0.39
2a*	CH_3		100	0.12
2b*	CH_3			19.8
3	CH_3		92	1.7
4	CH_2CH_3		87	3.2
5	CH_3		81	4.4
6	CH_3		83	4.6
7	CH_3		91	6
8	CH_3		80	6.2
9	CH_3		83	6.6
10	CH_3		97	9
11	CH_3		82	10
12	$-(CH_2)_4CH_2$			0.059
13	$-(CH_2)_5N^+(CH_3)_3$			0.18

TABLE I-continued

Compounds



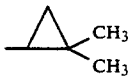
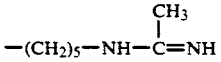
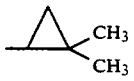
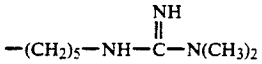
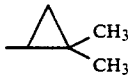
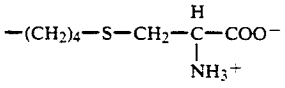
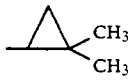
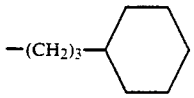
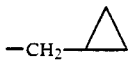
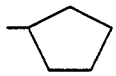
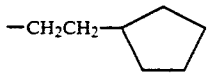
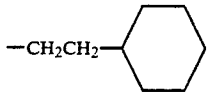
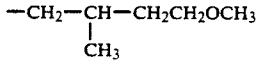
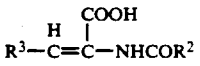
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
14	$-(CH_2)_5N^+(CH_3)_3$			1.11
15				0.72
16				0.89
17				0.21
18	CH_3	$-CH_2C(CH_3)_3$	75	20
19	CH_3	$-(CH_2)_6CH_3$	72	26
20	CH_3	$-(CH_2)_2CH_3$	69	30
21	CH_3		68	30
22	CH_3		64	22
23	CH_3	$(CH_2)_3CH_3$	64	32
24	CH_3		59	30
25	CH_3	$-(CH_2)_4CH(CH_3)_2$	57	
26	CH_3		56	
27	CH_3		54	
28	CH_3	$-CH_2-(CH_2)_3CH_3$	54	39
29	CH_3	$-(CH_2)_5CH_3$	49	
30	CH_3	$-CH(CH_2CH_3)CH_2CH_2CH_2CH_3$	33	
31	CH_3	$-CH(CH_2CH_2CH_3)_2$	13	
32	CH_3	$-CH(CH_3)_2$	31	
33	$HOO-CH_2CH_2$		90	5
34	CH_3		88	9
35	CH_3	$CH_2CH_2CH_2CH_2CH_2Br$	70	19
36	CH_3	$CH_2CH_2CH_2CH_2Cl$	64	20

TABLE I-continued

Compounds



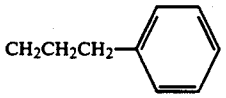
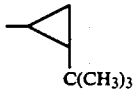
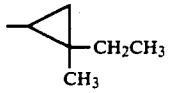
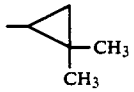
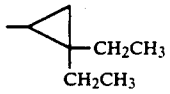
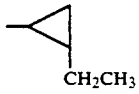
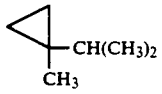
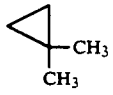
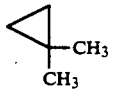
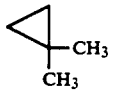
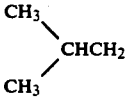
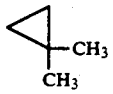
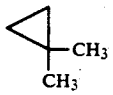
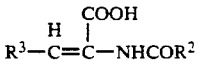
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
37	CH_3		72	11
38	CH_3		90	6.5
39	$CH_3(CH_2)_4$	$CH_2-CH(CH_3)_2$	95	2.6
40	CH_3		100	0.45
41	$(CH_3)_2CH$		98	0.54
42	CH_3		98	0.86
43	CH_3		96	1.6
44	CH_3		95	3
45	CH_3CH_2		98	0.18
46	Ph		100	0.62
47	$CH_3CH_2CH_2$		98	0.11
48			97	0.23
49	$CH_3(CH_2)_3$		100	0.11

TABLE I-continued

Compounds



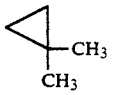
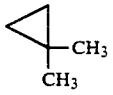
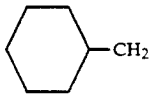
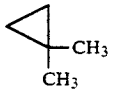
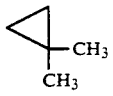
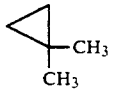
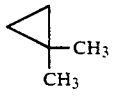
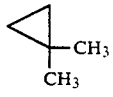
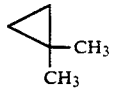
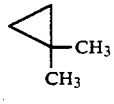
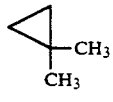
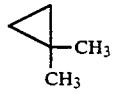
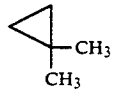
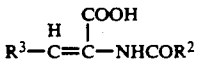
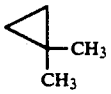
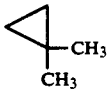
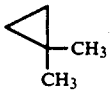
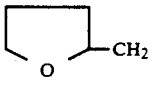
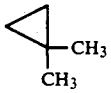
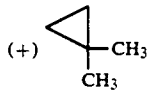
Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
50	$CH_3(CH_2)_4$		100	0.17
51	$HOOCCH_2CH_2$		98	0.145
52			100	0.15
53	$PhCH_2CH_2$		96	0.33
54	$CH_3SCH_2CH_2$		99	0.12
55	$CH_3SO_2CH_2CH_2$		96	0.5
56	$CH_3(CH_2)_5$		98	0.149
57	$CH_3(CH_2)_6$		99	0.092
58	$CH_3(CH_2)_9$		96	0.14
59	$PhCH_2$		98	0.44
60	$CH_3O(CH_2)_3$			0.28
61	$CH_3OCH_2CH_2$		98	0.32

TABLE I-continued

Compounds



Dipeptidase Inhibitor	R^3	R^2	% Inhibition at 10^{-4} M	K_i (μM)
62	$(CH_3)_3CCH_2$			0.34
63	$(CH_3)_2CHCH_2CH_2$		98	0.15
64	$H_2OC(CH_2)_3$		99	0.048
65				0.39
66	$CH_3(CH_2)_4$			.08

*Compounds 2, 2a, and 2b are the racemic, dextrorotatory and levorotatory forms respectively.

EXAMPLE 2

In Vivo Test Data

An in vivo assay on the mouse was conducted as follows: 20 g Charles River CD, female mice were injected subcutaneously with the chosen dose of the chemical inhibitor. About two minutes later, the dose of thienamycin was given intravenously. A control of thienamycin above was also conducted. The level of thienamycin in the urine as a % of dose was measured using a bioassay technique. Results are found in Table II. The two test compound numbers are those from Table I. Compound 7 is Z-2-isovaleramido-2-butenoic acid; compound 10 is Z-2-cyclopropylcarboxamido-2-butenoic acid.

TABLE II

Compound	Dose, mg/kg Compound	Dose, mg/kg Thienamycin	% Urinary Recovery of Thienamycin
7	50	10	53
7	10	10	53
10	50	10	56
Control	—	10	25–30

EXAMPLE 3

The compounds Z-2-isovaleramido-2-butenoic acid, Compound 7, and Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, compounds were studied, in more detail in vivo in combination with thienamycin (THM), in the mouse. The general test procedure was similar to that of Example 2. Results are summarized in Table III and Table IV.

TABLE III

Effect of Co-administered Z-2-Isovaleramidobutenoic Acid (Compound 7) on the Urinary Recovery of Thienamycin in the Mouse[a]

Route[b]		mg/kg Dose		Urinary Recovery
Compound 7	THM	Compound 7	THM	of THM, %
—	IV or SC	—	10	30 ± 5
SC	SC	0.3	10	33
SC	IV	2	10	42
SC	SC	2	10	47
SC	IV	10	10	53
SC	SC	50	10	54
SC	IV	50	10	53
SC	SC	80	10	59
SC	SC	100	10	81

[a]20 g Charles River, CD_1 female mice
[b]Co-administered

TABLE IV

Effect of Co-administered Z-2-(2,2-Dimethylcyclopropanecarboxamido)-butenoic acid (Compound 2) on Urinary Recovery of Thienamycin in the Mouse[a]

Route[b]		mg/kg Dose		Urinary Recovery
Compound 2	THM	Compound 2	THM	THM, %
—	SC	—	10	30 ± 5
SC	SC	0.1	10	35
SC	SC	0.3	10	40
SC	SC	1	10	46
SC	SC	10	10	60
SC	SC	30	10	73

[a]20 g Charles River, CD_1 female mice
[b]Co-administered

EXAMPLE 4

In another mouse study, the systemic antibacterial activity of thienamycin was enhanced approximately three-fold by coadministering Z-2-isovaleramido-2-butenoic acid, see Table V.

TABLE V

Effect of Co-administered Z-2-Isovaleramido-2-butenoic acid on the Systemic Efficacy of Thienamycin on the Treatment of *Staphalococcus aureus* Infections

		ED_{50}, mg/kg
THM	Alone	0.2
	+100 mg/kg inhibitor	0.06

EXAMPLE 5

A male beagle was used for a study of the effect of dipeptidase inhibitors on the urinary recovery of N-formimidoyl thienamycin. In a control study, the dog was given 5 mg/kg IV of the N-formimidoyl thienamycin without inhibitor. A second experiment used the same amount of N-formimidoylthienamycin, but also administered Z-2-isovaleramido-2-butenoic acid in 3 doses, each providing 20 mg/kg of the compound. The first dose was administered just after injection of the N-formimidoylthienamycin, the second at 40 min. and the third at 60 min. The third study employed a single dose (2 mg/kg) of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, administered just before injection of the N-formimidoyl thienamycin. The results are in Table VI.

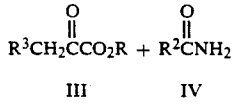
TABLE VI

Urinary Recovery 3 Hours Following the Administration of N-formimidoylthienamycin (5 mg/kg IV) in a Male Beagle

Test Compound	% Urinary Recovery
N-formimidoyl thienamycin	7.8
plus Z-2-isovaleramido-2-butenoic acid	46
plus Z-2-(2,2-dimethylcyclopropane carboxamido)-2-butenoic acid	53

SECTION 2. EXAMPLES ILLUSTRATING CHEMICAL PREPARATIONS

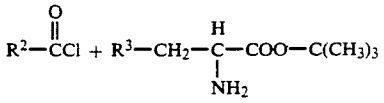
The inhibitor compounds are made by condensing directly the appropriate 2-keto acid or ester and an amide:



III IV I

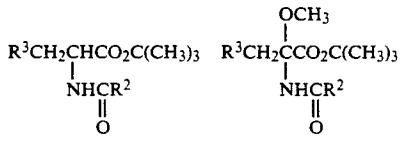
wherein R^2 and R^3 are as defined, and R is hydrogen or alkyl. The general reaction conditions involve mixing approximately 1–4:1 parts of the acid to the amide in an inert solvent such as toluene or methyl isovalerate and heating at reflux with azeotropic removal of water for from 3–48 hours, preferably 5–24 hours. The solution when cooled normally yields the product in crystalline form, but the product can also be isolated using a base extraction process. The product can be recrystallized by using generally known techniques. Condensations of keto esters require use of small amount of p-toluenesulfonic acid as catalyst. The catalyst also is helpful in some condensations with keto acids.

Another route to the novel inhibitor compounds uses an α-amino acid, t-butyl ester in reaction with an acid chloride:

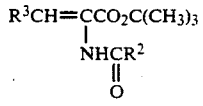


V VI

This reaction takes place in the presence of base, such as triethylamine, in a solvent such as methylene chloride. The resulting N-acylated product (VII) is then oxidized by treatment with t-butyl hypo-chlorite followed by addition of sodium methoxide. This yields the 2-methoxy derivative (VIII) and/or its elimination product, the α,β-unsaturated ester (IX). Further treatment with anhydrous hydrochloric acid converts either VIII or IX (or the mixture of both) to the desired α,β-unsaturated free acid (II).

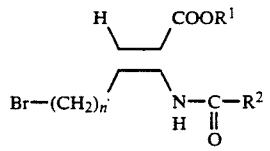


VII VIII



IX

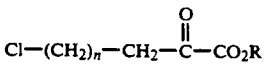
Some compounds wherein R^3 has a terminal substituent which is an amino, quaternary nitrogen, thio derivative, alkoxy, guanidino, acyloxy or cyano can be made most conveniently from an intermediate having a terminal bromine. In this case, the intermediate has the structure



X

wherein n is the number of carbons in the desired hydrocarbon chain (e.g., from 3–7). In order to prepare R^3 having a terminal trimethylammonium substituent, the bromo intermediate is reacted with trimethylamine; to yield the amino; the bromo intermediate is reacted with ammonia; the guanidino, reaction is with guanidine; to prepare the thio derivatives, including 2-amino-2-carboxyethylthio, the bromo compound is reacted with cysteine HCl, or the appropriate mercaptan. Derivatized amino, such as formamidino, ureido, and acylamido (acetamido) can be made from the compounds having an amino group by reacting with o-benzyl formimidate HCl, potassium cyanate and the appropriate acyl anhydride (acetic dehydride), respectively.

Another route for preparing compounds when R^3 is a terminally substituted thio derivative utilizes a chloro-keto ester intermediate



XI

in reaction with the desired amide,

IV

in toluene at reflux in the presence of a catalytic amount of p-toluene sulfonic acid. The resulting intermediate is hydrolyzed to the acid; the chloro group is then displaced in reaction with the appropriate mercaptan. This reaction is valuable since it permits use of the chiral amide IV, thereby preparing a functionalized side chain. In addition, the mixture of Z+E isomers prepared after the mercaptan condensation can be directly isomerized into the Z form by adding acid to a pH about 3, and heating to about 90° C. for 30 minutes. Only the Z form remains, and recovery is simple and straight forward.

EXAMPLE 6

Z-2-Isovaleramido-2-butenoic Acid

A solution of 1.07 g (10.5 mmole) of 2-ketobutyric acid and 0.71 g (7.0 mmole) of isovaleramide in 15 ml of toluene was stirred under reflux with collection of H_2O in a small Dean-Stark trap. After 5 hrs, the solution was cooled, resulting in fairly heavy crystallization. After standing, the solid was collected on a filter and washed with toluene and then with CH_2Cl_2. Yield of white crystals=0.47 g, mp 172°–174° (slight prelim. softening). The material was recrystallized from diisopropyl ketone. Tlc (4:1 toluene-AcOH) now showed only a faint trace of the other isomer. Yield of white crystals=0.32 g (25%), mp 175° (slight prelim. softening). NMR indicated essentially exclusively Z-isomer.

Anal. ($C_9H_{15}NO_3$)	Calcd.	Found
C	58.36	58.59
H	8.16	8.55
N	7.56	7.43

EXAMPLE 7

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-pentenoic acid

A solution of 1.74 g (15 mmole) of 2-ketovaleric acid and 1.13 g (10 mmole) of 2,2-dimethylcyclopropanecarboxamide in 20 ml of toluene was refluxed with stirring with collection of H_2O in a small Dean-Stark trap. After 20 hrs. the solution was cooled and treated with a gentle stream of N_2. Before much of the solvent had evaporated, crystallization was induced by scratching. After standing, the solid was collected on a filter and washed with toluene and some Et_2O. Yield of white crystals=0.63 g (30%), mp 154.5°–155.5° (slight prelim. softening). Tlc (4:1 toluene-AcOH) showed only an extremely faint trace of the other isomer. NMR was consistent with the Z-configuration.

Anal. ($C_{11}H_{17}NO_3$)	Calcd.	Found
C	62.53	62.86
H	8.11	8.27

-continued

Anal. ($C_{11}H_{17}NO_3$)	Calcd.	Found
N	6.63	6.75

EXAMPLE 8

Z-2-(3-Cyclopentylpropionamido)-2-butenoic acid

A solution of 1.41 g (10 mmole) of 3-cyclopentylpropionamide and 1.53 g (15 mmole) of 2-ketobutyric acid was stirred and refluxed under a small Dean-Stark trap. After 8 hrs. the solution was cooled, resulting in heavy crystallization. The solid was collected on a filter and washed with toluene and CH_2Cl_2. Yield of white crystals=1.44 g, mp 180.5°–182° (prelim. softening). The material was recrystallized from methyl ethyl ketone. Yield of white needles=0.63 g (28%), mp 184°–185° (slight prelim. softening). Tlc (4:1 toluene-AcOH) now showed a single spot, and NMR indicated essentially pure Z-isomer.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.97	63.99
H	8.50	8.67
N	6.22	6.27

EXAMPLE 9

Z-2-(2-Ethylhexanamido)-2-butenoic acid

10 g. of 2-ethylhexanoyl chloride was added dropwise with stirring to 25 ml of cold conc. NH_4OH solution, resulting in immediate precipitation. The mixture was allowed to stir for 2 hrs., then filtered, and air dried to give 6.5 g. of amide. 1.4 g (10 mmole) of the above compound and 1.5 g of ketobutyric acid (15 mmole) were refluxed in 25 ml toluene for 15 hrs with removal of water. The reaction mixture was cooled and partly evaporated with a stream of N_2. Crystallization of product occurred after standing for 3 hrs. The crystals were collected, washed 3× with toluene, and air dried. There was isolated 5 1 13 g (50%) of product, mp 160°–162° . NMR was in accord with the assigned structure and indicated <5% E isomer. Tlc (4:1 toluene-AcOH) showed a single spot.

Anal. ($C_{12}H_{21}NO_3$)	Calcd.	Found
C	63.40	63.63
H	9.30	9.43
N	6.16	5.88

EXAMPLE 10

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-butenoic acid

1.53 g (15 mmoles) of 2-ketobutyric acid, 1.13 g (10 mmoles) of 2,2-dimethylcyclopropanecarboxamide and 20 ml of toluene stirred at reflux for 10 hours. After cooling the crystalline solid was filtered and washed with toluene (3×10 ml) and dried to give 1.06 g of product, mp 140°–141° C. Tlc (4:1 toluene-AcOH) showed essentially one spot and the NMR spectrum fit the desired structure.

Recrystallization from EtOAc gave after drying 0.533 g of product mp 142°–143.5°, homogeneous by tlc.

Anal. ($C_{10}H_{15}NO_3$)	Calcd.	Found
C	60.90	60.92
H	7.67	7.71
N	7.10	7.38

EXAMPLE 11

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenedioic acid

A mixture of 1.0 g. of 2,2-dimethylcyclopropanecarboxamide, 2.4 g. of 2-ketoadipic acid and 25 ml. of methyl isovalerate was heated under reflux for 4 hrs, with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). After standing at room temperature overnight, the crystalline precipitate was filtered, washed with ether and recrystallized from ethyl acetate to give 0.23 g. of product, m.p. 163°–165°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{17}NO_5$)	Calcd.	Found
C	56.46	56.20
H	6.71	6.83
N	5.49	5.32

EXAMPLE 12

Z-2-(2,2-Diethylcyclopropanecarboxamido)-2-butenoic acid

A mixture of 2.3 g of 2-ketobutyric acid, 2.0 g of 2,2-diethylcyclopropanecarboxamide, and 25 ml of toluene was heated under reflux for 16 hrs with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). No product precipitated upon cooling. Ether (25 ml) was added and the mixture was extracted with saturated $NaHCO_3$ (3 times). The combined extracts were acidified with concentrated HCl. The gummy precipitate crystallized when triturated with water. Recrystallization from ethyl acetate gave 0.31 g of product, m.p. 129°–30°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.98	64.01
H	8.50	8.62
N	6.22	6.21

EXAMPLE 13

2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenoic acid

Step A: DL-Norleucine t-butyl ester

General procedure of R. Roeske, *J. Org. Chem.* 28, 1251 (1963).

To a suspension of 9.82 g (75 mmole) of DL-norleucine in 80 ml of dioxane in a 500 ml. pressure bottle cooled in an ice bath was added slowly (with swirling) 8 ml of concentrated H_2SO_4. The resulting mixture was cooled in a dry ice bath as 80 ml of liquid isobutylene was added. The mixture was allowed to warm to room temperature and shaken under autogenous pressure for ~23 hrs. After most of the isobutylene had been vented off, the slightly hazy solution was cooled in ice and then added to a cold mixture of 400 ml of 1N NaOH and 500 ml of Et_2O. After shaking in a separate funnel, the layers were separated, and the aqueous fraction was washed with an additional 100 ml of Et_2O. The Et_2O solution was shaken with 150 ml of 0.5 N HCl. The acidic aqueous fraction was treated with 2.5 N NaOH until strongly basic and then shaken with 250 ml. of Et_2O. The Et_2O solution was dried ($MgSO_4$), filtered, and concentrated on the rotovac. After prolonged pumping on high vacuum over a steam bath, final yield of clear, colorless residual oil = 9.04 g (65%). NMR now showed only a trace of dioxane. TLC (9:1 $CHCl_3$-MeOH) showed a single spot.

Step B N-(2,2-Dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester

To a solution of 8.98 g (48 mmole) of DL-norleucine t-butyl ester and 5.05 g (50 mmole) of triethylamine in 100 ml of CH_2Cl_2 stirred in an ice bath under a drying tube was added dropwise (over a period of 75 min.) a solution of 6.39 g (48 mmole) of 2,2-dimethylcyclopropanecarbonyl chloride (M. Elliot and N. R. James, British Patent No. 1,260,847 (1972)) in 50 ml of CH_2Cl_2. Precipitation of Et_3N HCl occurred during the addition, especially toward the end. As the ice gradually melted, the mixture was allowed to warm to room temperature. After 16 hrs, the mixture was shaken with 200 ml of 0.5 N HCl. The CH_2Cl_2 fraction was washed with an additional 200 ml of 0.5N HCl, then with 2×200 ml of 0.5 N NaOH, and finally 200 ml of H_2O. The CH_2Cl_2 fraction was dried with $MgSO_4$, treated with charcoal, and filtered through Celite. The filtrate was concentrated on the rotovac (finally under high vacuum). Yield of light orange residual oil = 11.93 g (88%). Tlc (2:1 hexane-EtOAc) showed a single spot. NMR and IR were in accord with the assigned structure. After standing for several days, the unused porition of this material crystallized: m.p. 52°–>65°.

Step C: t-Butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate

Based on procedure of H. Poisel and V. Schmidt, *Chem. Ber.*, 108 2547 (1975).

To a solution of 6.37 g (22.5 mmole) of N-(2,2-dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester in 35 ml of Et_2O stirred at room temperature under N_2 in the dark was added 2.69 ml (2.45 g, 22.5 mmole) of t-butyl hypochlorite. After 15 min., a solution of sodium methoxide prepared by dissolving 0.52 g (22.6 mmole) of sodium in 35 ml of MeOH was added. Stirring was continued at ambient temperature under N_2 in the dark. After 16.5 hrs., the precipitated NaCl was filtered off. The filtrate was diluted with Et_2O and washed successively with 3×50 ml of 0.5 N HCl, 50 ml of saturated Na_2CO_3, and 2×50 ml of H_2O. The Et_2O phase was dried over $MgSO_4$ and filtered. The filtrate was concentrated on the rotovac. The pale, golden-yellow residual oil (6.45 g) was subjected to preparative high pressure liquid chromatography, resulting in the separation and isolation of 273 mg and 496 mg of the two diastereomers of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate (respective mp's 114°–118° and 124°–125.5°) as well as 1.97 g of a single isomer (apparently Z) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate (color-less oil).

Step D: 2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenoic acid

A solution of 0.84 g (3.0 mmole) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate in 10

ml of Et_2O saturated with anhydrous HCl was allowed to stand at room temperature under a drying tube. After 17 hrs, the solution was evaporated, and the residual gum was dissolved in 10 ml of saturated $NaHCO_3$. This solution was washed with an additional 15 ml of 0.5 N HCl, then dried ($MgSO_4$), filtered, and concentrated to give a viscous oil. The oil was crystallized from toluene. Yield of white crystals =0.32 g (47%), m.p. 119°–122°. TLC (4:1 toluene-AcOH) showed a single spot. NMR indicated essentially pure Z-isomer. (Note: Treatment of the methanol adduct, t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexenoate, with anhydrous HCl in Et_2O under similar conditions gave the same product.)

EXAMPLE 14

(+)-Z-2-(2,2-Dimethylcyclopropanecarbonylamino)-2-octenoic acid, sodium salt

The reagents, (+)-2,2-dimethylcyclopropanecarboxamide, 7.0 g.; 2-keto-octanoic acid ethyl ester, 14.7 g.; 50 mg. of p-toluene sulfonic acid; and 100 ml. of toluene was changed to a 250 ml. three-necked flask under a Dean Stark trap containing several molecular sieve pellets. The mixture was refluxed vigorously for 27 hours. The resultant light yellow solution was cooled and concentrated in vacuo, at a water bath temperature of 45° C., in the presence of water to help remove toluene. The gummy residue was suspended in 230 ml. of 2N NaOH and stirred at 30° C. for 3 hours; then the temperature was raised to 35° C. for an additional 2½ hrs. until a clear solution formed. The solution was then cooled, 85 ml. methylene chloride added, and the pH adjusted to 8.5 using 4N HCl with stirring. The organic layer was separated and discarded. The aqueous layer (366 ml.) was assayed by liquid chromatography to contain 37.2 mg/ml; 87% Z isomer. Another 85 ml. portion of CH_2Cl_2 was then added and pH adjusted to 4.5 with stirring. The organic layer was separated and the aqueous layer reextracted with 50 ml. of CH_2Cl_2, with the pH again adjusted to 4.5. Combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to a gum. This residue was dissolved in 150 ml. isopropanol and 15 ml. water and the pH adjusted to 8.2 with 2N NaOH. The resulting solution was concentrated to an oily residue which was flushed with isopropanol until it turned to a crystalline solid, indicating that most water had been removed. It was crystallized from 120 ml. of isopropanol, (cooled in ice for 1 hour) filtered, and washed with 50 ml. cold isopropanol followed by copious amounts of acetone. It was dried at 60° C./0.1 mm/2 hours to yield 10.74 g (63.2%) crystalline material, having essentially a single peak in liquid chromatography, m.p. 241°–243° C.

The starting material, (+)-2,2-dimethylcyclopropanecarboxamide is most conveniently prepared by resolution of the D,L acid, followed by reaction with oxalyl chloride and then ammonia to give the resolved amide.

One way of making the starting material is as follows: 23.1 g. of D,L-2,2-dimethylcyclopropanecarboxylic acid was suspended in 33 ml H_2O and the pH adjusted to 8.0, using 50% NaOH, about 10 ml. To this was added a solution of 38.4 g quinine in a mixture of 60 ml. methanol and 30 ml. H_2O to which had been added about 8 ml of concentrated HCl in another 30 ml. H_2O to give a pH of 7.1. (This was actually a solution of quinine hydrochloride.)

These solutions were added all at once, with stirring. The gummy crystalline material which formed was heated to give two clear layers and again stirred vigorously while cooling to give a crystalline product. This product was permitted to stand over two days at room temperature. It was then filtered, washed with 2×10 ml water, and 2×10 ml 50% methanol, and air dried with suction. The yield of crude quinine salt was 44.8 g (48.7% yield) monohydrate, m.p. 113°–116° C., having a $[\alpha]_D^{20}$ of −94.3°, C=1.0; $CHCl_3$. This material was recrystallized from acetone to yield 24.35 g, m.p. 127°–130° C. This purified quinine salt was converted to the acid by reaction with aqueous base and chloroform, followed by acid, to yield (96%) 3.9 g having $[\alpha]_D^{20}$ of +146.0°.

This acid was converted to the amide as follows: A charge of 30.5 g (+)acid was added over 5–10 minutes through a dropping funnel to chilled (10° C.) oxalyl chloride, 54 ml., containing 1 drop dimethylformamide. This was stirred overnight at ambient temperature. A clear solution was observed, which was added to 100 ml. methylene chloride to dilute. Excess oxalyl chloride was removed by concentrating and the mixture flushed twice with methylene chloride.

The resultant solution was diluted with an equal volume of methylene chloride, and added continuously through a dropping funnel to about 100 ml. anhydrous liquid ammonia which was diluted with 100 ml methylene chloride. A dry ice-acetone cooling bath was used during the addition. When all was added, the cooling bath was removed and the mixture stirred at room temperature for about ½ hour. The mixture was filtered, to remove precipitated ammonium chloride, and concentrated to dryness. The crude weight was 26.6 g. (88%). excess hot ethyl acetate and filtered through a preheated sintered glass funnel to separate from trace NH_4Cl. Excess ethyl acetate was atmospherically distilled off. When half the volume remained, 130 ml of heptane were added, and ethyl acetate was continued to be distilled off, until the boiling point started to rise (to near 80° C.; much of product had already crystallized out). Heat was removed, and the mixture let cool gradually to about 30° C., then cooled with an ice bath to 0°–5° C. for about ½ hour. The product was recovered as nice silvery-white crystalline flakes, washed with 3× ethyl acetate/hexane mixture, 1/1.5 and air dried to constant weight. It weighed 23.3 g (77.1% yield overall, 87.6% recovery from crude), m.p.=135°–138° C. (varies with rate of heating). Angle of rotation was determined by dissolving 0.0543 g in 10 ml chloroform, $[\alpha]_D^{20}$ = +100.9°.

EXAMPLE 15

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

Step A: 2,2-Dichlorocyclopropanecarboxamide

A 7.1 g sample of 2,2-dichlorocyclopropanecarbonyl chloride (U.S. Pat. No. 3,301,896, issued Jan. 31, 1967) was added dropwise to 75 ml of concentrated ammonium hydroxide with vigorous stirring. The temperature of the reaction mixture was maintained below 10° C. with an ice bath. The mixture was stirred in the ice bath for 30 min., then at room temperature for 1 hr. The aqueous ammonia was evaporated under reduced pressure (bath at 50° C.). The solid residue was extracted with hot ethyl acetate (3×30 ml). The extracts were boiled down to 40 ml and 20 ml of hexane was added.

After cooling in ice, the solid was filtered, washed with ethyl acetate-hexane (1:1) and dried to give 2.7 g of 2,2-dichlorocyclopropanecarboxamide, m.p. 144–146°. The NMR spectrum was in accord with the desired structure.

Anal. ($C_4H_5Cl_2NO$)	Calcd.	Found
C	31.20	31.26
H	3.27	3.31
N	9.10	9.11
Cl	46.04	45.79

Another 1.3 g of amide, m.p. 143°–145° could be recovered from the mother liquor.

Step B: Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

A mixture of 1.53 g (15 mmoles) of 2-ketobutyric acid, 1.54 g (10 mmoles) of 2,2-dichlorocyclopropanecarboxamide and 10 ml of toluene was heated under reflux for 12 hrs. with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). An additional 0.7 g of 2-ketobutyric acid was added and the reaction mixture was heated under reflux for an additional 12 hrs. The mixture was cooled, diluted with 20 ml of toluene and extracted with saturated sodium bicarbonate (3×10 ml). The extracts were combined, washed with ether and acidified to pH 3 (pH meter) with concentrated hydrochloric acid. A gum precipitated which soon solidified. It was filtered, washed with water, dried and recrystallized from nitromethane to give 423 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-butenoic acid, m.p. 188°–189.5° C. The NMR spectrum was in accord with the desired structure.

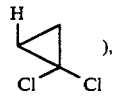
Anal. ($C_8H_9Cl_2NO_3$)	Calcd.	Found
C	40.36	40.48
H	3.81	3.80
N	5.88	5.91
Cl	29.78	29.53

EXAMPLE 16

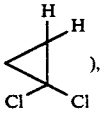
Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-octenoic acid

A mixture of 1.19 g (7.5 mmoles) of 2-ketooctanoic acid, 0.77 g (5.0 mmoles) of 2,2-dichlorocyclopropanecarboxamide, and 5 ml toluene were reacted using the same procedure as in the previous example. The crude product (537 mg) was purified by conversion to the methyl ester (BF_3/CH_3OH), preparative TLC (silica gel G, 4:1 hexane-EtOAc) and saponification of the pure Z-methyl ester (0.3M LiOH/CH_3OH) to give 88 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid as a partially crystalline gum. NMR spectrum (DMSO-d_6): δ9.68 (s, 1H, NH), 6.50 δ (t, 1H,

2.83δ (t, 1H,



1.97 δ (d, 2H



0.87 δ (t, 3H, CH_3).

EXAMPLE 17

Z-8-Bromo-2-(2,2-Dimethylcyclopropanecarboxamido)-2-octenoic acid

To a suspension of 14.4 g (0.3 mole) of 50% NaH dispersion in 360 ml of toluene cooled in an ice bath and in a N_2 atmosphere was added over 45 min. a solution of 146 g (0.6 moles) of 1,6-dibromohexane and 57.6 g (0.3 mole) of ethyl 1,3-dithiane-2-carboxylate in 120 ml of DMF. The cooling bath was removed and the mixture stirred at room temperature for 20 hrs. The reaction mixture was washed with water (3×210 ml), dried over $MgSO_4$ and evaporated under reduced pressure to give 179.5 g of a yellow oil containing the desired alkylated dithiane, 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

To a suspension of 426 g (2.4 moles) of N-bromosuccinamide in 800 ml of acetonitrile and 200 ml of H_2O was added over 45 min. a solution of the crude dithiane in 100 ml of acetonitrile. The temperature of the reaction mixture was maintained below 25° C. with an ice bath. After stirring at 20° C. for 10 min. the dark red reaction mixture was poured into 2 l. of hexane-CH_2Cl_2 (1:1). The solution was shaken with saturated $NaHSO_3$ (2×400 ml) and water (1×500 ml). Then 400 ml of saturated Na_2CO_3 solution was added in small portions (vigorous CO_2 solution). After the foaming subsided the funnel was shaken and the aqueous phase separated. The organic layer was extracted with saturated Na_2CO_3 solution (400 ml) and water (500 ml) and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave 133.8 g of crude bromo ketoester containing 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

A mixture of 133.8 g of crude bromo ketoester, 133 ml of 50% hydrobromic acid and 267 ml of acetic acid was heated at 90° C. (internal temperature) for 75 min. The dark solution was evaporated under reduced pressure until most of the acetic acid was removed. The residue was dissolved in 500 ml of ether, washed with water (2×100 ml) and extracted with saturated $NaHCO_3$ (3×200 ml). The combined $NaHCO_3$ extracts were extracted with ether (2×100 ml) and acidified with concentrated HCl. The precipitated oil was extracted with ether (3×200 ml). The ether extracts were washed with water (1×100 ml) and saturated brine (1×100 ml) and dried over $MgSO_4$. Removal of the ether under reduced pressure gave 46.2 g of pure bromoketo acid. Homogeneous by TlC (silica gel, 4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired product.

A mixture of 46.1 g (0.194 moles) of the bromoketo acid, 17.6 g (0.156 mole) of 2,2-dimethylcyclopropanecarboxamide and 450 ml of toluene was heated under reflux for 13 hrs., with collection of water in a small Dean-Stark trap. After cooling, the clear reaction mixture was extracted with saturated $NaHCO_3$ solution (4×100 ml). The combined extracts were washed with ether (2×100 ml) and then the pH was adjusted to 3.5 (pH meter) by addition of concentrated HCl. An oil precipitated which soon crystallized. The solid was filtered, washed well with water and dried. Recrystallization from acetonitrile gave 22.5 g of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, m.p. 151°–153° C. Homogeneous by TLC (4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired structure.

Anal. ($C_{14}H_{22}BrNO_3$)	Calcd	Found
C	50.61	50.66
H	6.67	6.96
N	4.22	4.45
Br	24.05	23.95

The following ω-bromo compounds were prepared using the same procedure:

Z-6-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-7-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

Z-9-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid;

Z-10-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid;

Z-8-Bromo-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid.

EXAMPLE 18

Z-8-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid

A solution of 664 mg (2 mmoles) of Z-8-bromo-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 10 ml of 40% aqueous dimethylamine was allowed to stand at room temperature for 4 hrs. The solution was poured onto a 3.5×20 cm column of Dowex 50W-$_x$8 (100–200 mesh, H+-) ion exchange resin and the column eluted with water until the effluent was no longer acidic (~200 ml). The column was then eluted with 300 ml of 2N ammonium hydroxide. The effluent was evaporated under reduced pressure to give 600 mg of a colorless glass. This material was dissolved in 3 ml of ethanol, filtered, and added dropwise to 200 ml of rapidly stirred acetone. A gummy solid precipitated which crystallized upon stirring for two days. The solid was filtered, washed with acetone, and dried to give 445 mg of Z-8-dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid as colorless, hygroscopic crystals, m.p. 101°–112° C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{16}H_{28}N_2O_3.H_2O$)	Calcd.	Found
C	61.12	61.03
H	9.62	9.28
N	8.91	8.67

The following ω-amino derivatives were prepared using essentially the same procedure.

Z-10-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid;

Z-8-Amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-Dimethylamino-2-(2,2-dimethylcylclopropanecarboxamido)-2-heptenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-7-(N-methylpiperazinyl)-2-heptenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-pyrrolidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(N-methylpiperazinyl)-2-octenoic acid;

Z-8-Allylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

(2,2-dimethylcyclopropanecarboxamido)-8-piperidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-propargylamino-2-octenoic acid;

Z-8-N-[1-Deoxy-(1-methylamino)-D-glucityl]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-(1-Adamantylamino)-2-(2,2-dimethylcyclopropanecarboxamido-2-octenoic acid;

Z-8-Diallylamino-2-(2,2-dimethylcyclopropanecarboxamido-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(2-hydroxyethylmethylamino)-2-octenoic acid;

Z-8-[(Carboxylmethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-diethylamino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[tris(-hydroxymethyl)methylamino]-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-10-(N-methylpiperazinyl)-2-decenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)ethylamino-]2-octenoic acid;

EXAMPLE 18 A

Z-8-[(Carboxymethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid

3.32 g. of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, 1.0 g. of $CH_3NHCH_2CO_2H$, 3.5 g. of Na_2Co_3 and 30 ml of water were heated at 80° C. in N_2 for 1.5 hours. After purification, 1.0 g. of product was obtained, calc. for $C_{17}H_{28}N_2O_5.2H_2O$:C, 54,24; H, 8.57; N, 7.44; found: C, 54.40; H,8.34; N, 7.16.

EXAMPLE 18 B

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)ethylamino]-2-octenoic acid

Was prepared by reacting the same bromo intermediate (335.1 mg) with 138.2 mg 1-aminoethane phosphoric acid, and 435 mg Na_2CO_3 in 5 ml water, following essentially the same procedure, Ki=0.16.

EXAMPLE 19

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid

A stream of CH_3SH gas was bubbled through a solution of 162 mg (3 mmoles) of sodium methoxide in 5 ml of methanol for 10 min. with cooling in an ice bath. The

solution was allowed to warm to room temperature and 332 mg (1 mmole) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was added. The solution was heated under reflux for 30 min. in a N_2 atmosphere. Most of the methanol was evaporated under reduced pressure, the residue was dissolved in 10 ml of water and acidified with 2.5 N HCl. The precipitated oil was extracted with ether (3×). The ether extracts were washed with water, saturated brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 178 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid, m.p. 82°–84° C. Homogeneous by TLC (toluene-acetic acid, 4:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}NO_3S$)	Calcd.	Found
C	60.18	60.36
H	8.42	8.68
N	4.68	4.59
S	10.69	10.87

The following compounds were prepared by similar methods.

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ethoxythiocarbonylthio-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(1-methyl-5-tetrazolylthio)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-7-{[(methoxycarbonyl)methyl]thio}-2heptenoic acid;

Z-8-Acetylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-[(2-Amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

6-(L-2-carboxethylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-8-(Carbomethoxymethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-6-(Carbomethoxymethylthio-2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoic acid;

Z-2-(2,2-dimethylcycloproopanecarboxamido)-6-(phosphonomethylthio-2-hexenoic acid.

The compound 7-(L-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid is prepared in a similar fashion as the above example, except that Z-7-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid (prepared as in Example 17) (185 mg, 1.05 mmoles) is dissolved in 2.02 ml NaOH solution (2.0 N), and deoxygenated by bubbling a stream of nitrogen gas through it for a minute. Then cysteine.HCl (185 mg, 1.05 mmoles) is added all at once and the reaction stirred at room temperature in a N_2 atmosphere for 3 hours. The reaction mixture is applied to 2×20 cm column of Dowex 50×4 (100–200 mesh H^+), and eluted with 300 ml H_2O), then 200 ml of 2N NH_3 solution. Ammonia evaporated under reduced pressure to give 284 mg of a yellowish glass. This product is dissolved in 4 ml ethanol, and the insoluble material filtered. The filtrate is added dropwise to rapidly stirred diethylether (150 ml). The solid which precipitates is filtered, washed with ether and dried to yield 171 mg product, having one spot (ninhydrin positive) in TLC (nBuOH, HOAc, H_2O; 4:1:1) rf. about 6; NMR is consistent with the desired structure.

Anal. ($C_{16}H_{26}N_2O_5S$)	Calcd.	Found
C	53.61	52.55
H	7.31	7.40
N	7.81	7.89
S	8.94	9.63

EXAMPLE 19 A

Sodium
Z-7-(L-amino-2-Carboxethylthio)-2-(2,2-dimethyl cyclopropane carboxamido)-2-heptenoic acid

A. Grignard Preparation of Ethyl-7-chloro-2-oxoheptanoate

Equimolar amounts (8 moles each) of 1-bromo-5-chloropentane and magnesium are reacted in tetrahydrofuran (960 ml) at 25° C. The flask is charged with mg. in the THF and the bromochloropentane added over 1 hr, then aged 2 hrs. After the reaction was judged complete, the reaction solution was added (cooled to −15° C.) to 16 moles of diethyloxalate in 1856 ml tetrahydrofuran, while maintaining the temperature at −10° C. 3 N.HCl was added to quench, keeping the temperature below 25° C. After stripping solvents, the calculated yield is 48.8% of the ethyl-1-chloro-6-oxoheptenoate.

B. Condensation and Hydrolysis

S-2,2-dimethylcyclopropyl carboxamide (1017 g), 2143.6 g of ethyl-7-chloro-2-ketoheptanoate, 9 liters of toluene and 12 g of p-toluene sulfonic acid were charged to a 22 L. flask, and heated to reflux with stirring. After 23 hrs., liquid chromatography showed the expected product ratio, and 4 L. of toluene were removed under slightly reduced pressure. The pot was charged with water, neutralized to pH 7 with 2N NaOH, and vacuum distilled leaving a final pot volume of about 5 liters.

This was hydrolyzed by adding 1760 g of 50% aq. NaOH (4 liters water) and stirring overnight. The flask was charged with 4 L. methylene chloride, and pH adjusted to 8.8 using HCl. unreacted amide crystallized out. The organic layers were separated from water, and then evaporated. The gummy residue was dissolved in 8 liters water containing 720 g 50% NaOH, and to this solution was charged 1818 g L. cysteine $HCl.H_2O$, 2 kg ice, 2484 g 50% NaOH and 1 liter water.

The pH of this solution, after aging overnight at room temperature, is adjusted to 3.0 with conc. HCl, and the resulting gummy suspension heated to 95° C. to afford a clear solution. After 30 minutes, no E isomer could be detected by lc. After work-up and purification, the overall yield was 50%. This material was recrystallized from acetonitrile. 1500 g of the recrystallized material was dissolved in 6 liters water and 910 ml 3.88N NaOH, then neutralized to pH 7, and lyophilized to afford 1569 g (98.6%) of the title compound; Analysis: calcd: C, 50.52; H, 6.62; N, 7.36; S, 8.43; Na, 6.04; found: C, 50.71; H, 6.78; N, 7.49; S, 8.52; Na, 5.92.

EXAMPLE 19 B

Z-8-[(2-Amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid was also prepared in a similar manner, to that described in Example 19, above, using 3.3 gm of the bromo intermediate, 1.3 g of $H_2NC(=O)$ CH_2SH, in 50 ml methanol 1.6 g of product, mp 127°–128° C. was obtained.

EXAMPLE 20

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt

A solution of 996 mg (3 mmoles) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 15 ml of 25% aqueous trimethylamine was allowed to stand at room temperature for 3 hrs. The reaction mixture was poured onto a 2×25 cm column of IRA-410 (50–100 mesh, OH^-) ion exchange resin and eluted with water until the effluent was no longer basic. The effluent was evaporated under reduced pressure to give 800 mg of a colorless glass. This material was dissolved in 20 ml of ethanol, filtered and diluted with 600 ml of acetone. After standing at room temperature overnight the crystalline solid which deposited was filtered, washed with acetone and dried to give 720 mg of Z-2-(2,2-dimethylcyclopropanecarboxamide)-8-trimethylammonium hydroxide-2-octenoic acid inner salt as hygroscopic crystals, m.p. 220°–222° C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{17}H_{30}N_2O_3$)	Calcd	Found
C	65.77	65.78
H	9.74	9.98
N	9.02	8.92

Other quaternary derivatives were prepared using essentially the same procedure; these are

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-pyridinium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(2-hydroxyethyldimethylammonium hydroxide)-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-trimethylammonium hydroxide-2-decenoic acid inner salt;

Z-10-(Benzyldimethylammonium hydroxide)-2-(2,2-dimethylcyclopropanecarboxamido)-2-decenoic acid inner salt;

Z-8-(Benzyldimethylammonium hydroxide)-2-(2,2dimethylcyclopropanecarboxamido)-2-decenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-9-trimethylammonium hydroxide-2-nonenoic acid inner salt;

Z-8-(2-Dimethylaminoethyldimethylammonium hydroxide)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid inner salt;

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

EXAMPLE 21

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid

A 350 mg sample of Z-8-amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was dissolved in 10 ml of water and the pH adjusted to 8.5 with 2.5N NaOH. A total of 947 mg of benzyl formimidate hydrochloride was added at room temperature in small portions over 20 min. while the pH was maintained between 8–9 by addition of 2.5N NaOH. After stirring at room temperature for 30 min., the cloudy reaction mixture was extracted with ether (3×) and applied to a 2×2.5 cm column of an AG50W-X4 (Na^+, 200–400 mesh) resin. After elution with water, the fractions containing the product were pooled and evaporated under reduced pressure. This material was dissolved in water and applied to a 2×25 cm column of an AGlX8 (HCO_3^-, 200–400 mesh) resin. After elution with water, the fractions containing pure product were pooled and evaporated under reduced pressure. The residue was dissolved in a few ml of warm ethanol, filtered, and added dropwise to 200 ml of ether with rapid stirring. Filtration and washing with ether gave 243 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid as an amorphous solid. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}N_3O_3 \cdot \frac{1}{2}H_2O$)	Calcd.	Found
C	59.69	60.04
H	8.59	8.64
N	13.92	13.57

The following amidino compounds were prepared using similar procedures:

Z-8-Acetamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-8-N-Benzylformamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-formamidino-2-decenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-(2-imidazolinyl-amino)-2-octenoic acid.

EXAMPLE 22

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid

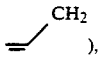
To a solution of 2 mmoles of guanidine (prepared from 432 mg of guanidine sulfate and 630 mg of barium hydroxide octahydrate) in 7 ml of water was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, and the solution was heated at 70° C. in a nitrogen atmosphere for 1 hr. The reaction mixture was applied to a 2×25 cm column of Dowex 50W-X8 (H^+, 100–200 mesh). After elution with water the fractions containing the product were pooled and evaporated under reduced pressure. The residue was dissolved in several ml of warm ethanol and added dropwise to 100 ml of ether with rapid stirring. Filtration and washing with ether gave 107 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid as an amorphous electrostatic powder. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). NMR (D_2O, NaOD): 6.48δ (t, 1H,

);

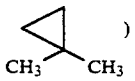
3.10δ (m, 2H,

),

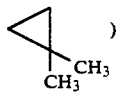
2.10δ (m, 2H,



1.17δ (s, 3H,



1,12 (S, 3H,



The following guanidino compound was prepared using the same procedure:

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(N,N-dimethylguanidino)-2-octenoic acid.

EXAMPLE 23

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic acid

To a solution of 2.43 mmoles of sodium methoxide in 5 ml of methanol was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid. The solution was heated under reflux in a nitrogen atmosphere for 1 hr. The reaction mixture was evaporated under reduced pressure, the residue dissolved in water and acidified with 2.5N hydrochloric acid. The oil which precipitated was extracted with ether (3×). The ether extracts were washed with water, and saturated brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 140 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic acid, m.p. 71°–72° C. Homogeneous by TLC (toluene-HOAc, 4:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}NO_4$)	Calcd.	Found
C	63.58	63.54
H	8.89	9.12
N	4.94	5.16

Using similar procedures, the following compounds were prepared:

Z-8-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-7-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;

Z-9-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-7-sulfo-2-heptenoic acid sodium salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-sulfo-2-octenoic acid sodium salt;

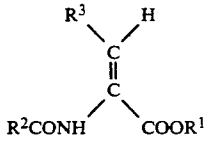
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-hydroxy-2-octenoic acid;

Z-8-Acetoxy-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

The Z-8-cyano-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic compound was prepared from 332 mg 8-bromo-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid and 100 mg NaCN in 2 ml DMSO, heated at 80° C. for 30 min. After extraction and purification, 102 mg of a colorless solid, mp 99°–103° C. were recovered, analysis for $C_{15}H_{22}N_2O_3$: Calcd: C, 64.73; H, 7.97; N, 10.06; Found C, 64.69; H, 8.14; N, 9.41.

What is claimed is:

1. A compound of the formula



R1 is hydrogen or a pharmaceutically acceptable cation;

R2 is X or Y

wherein

X is unsubstituted or substituted branched or linear alkyl of three to ten carbon atoms wherein a non-terminal methylene can be replaced by oxygen, sulfur or SO2, where said substituents are selected from the group consisting of halogen or cycloalkyl of three to six carbon atoms, with the proviso that, when said alkyl is substituted by said cycloalkyl, X is not more than ten total carbon atoms, with the further proviso that not more than six hydrogens of said alkyl can be substituted by said halogen, and with the further proviso that the carbon adjacent to the carbonyl cannot be tertiary;

Y is cycloalkyl of three to six carbon atoms, unsubstituted or substituted with one or two substituents where said substituents are selected from the group consisting of halogen or alkyl of one to four carbon atoms, with the proviso that, when said cycloalkyl is substituted by said alkyl, Y is not more than ten total carbon atoms;

R^3 is unsubstituted or substituted two to fifteen carbon alkyl wherein said substituent is halogen, and wherein a non-terminal methylene can be replaced by oxygen, sulfur or SO_2 and wherein the terminal carbon of said alkyl can be substituted by a moiety selected from the group consisting of amino, ureido, amidino, guanidino, one to four carbon alkylamino, dialkylamino of one to four carbons per alkyl substituent, trialkylammonium, quaternary hydroxyalkyldialkylammonium, acylamino, phosphonylalkylamino, hydroxyalkylamino, formamidino, alkylamidino, N,N-dialkylguanidino, hydroxyl, alkylcarbonyloxy, alkoxycarbonyl, carbamoyl, N,N dialkylcarbamoyl, thiol, acylthio, carboxy, phosphono, cyano, L-2-amino-2-carboxyethylthio or N-methyl-N-carboxymethylamino, with the proviso that no more than six hydrogens of said one to fifteen carbon alkyl can be substituted by halogen, with the further proviso that when R^3 is straight chain lower alkyl of one to four carbon atoms, R^2 cannot be straight chain lower alkyl of one to four carbon atoms, with the further proviso that the compound of the structural formula given above has the Z stereoconfiguration.

2. The compound of claim **1** in which R^2 is 2,2-dimethylcyclopropyl.

3. The compound of claim 1 in which R^2 is 2,2-dichlorocyclopropyl.

4. The compound of claim 1 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-[1-(phosphono)-ethylamino]-2-octenoic acid.

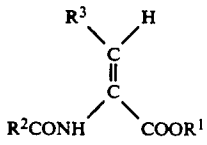
5. The compound of claim 1 which is Z-8-[(carboxymethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

6. The compound of claim 1 which is Z-8-[(2-amino-2-oxoethyl)thio]-2-(2,2-dimethylcyclopropane-carboxamido)-2-octenoic acid.

7. The compound of claim 1 which is Z-8-cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

8. The compound of claim 1 which is Z-8-acetamido-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

9. A compound of the formula



R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl;

R^1 is hydrogen, loweralkyl of 1–6 carbon atoms, dialkylaminoalkyl, or a pharmaceutically acceptable cation; R^3 is a hydrocarbon chain of 3–7 carbon atoms unsubstituted or substituted with a terminal substituent taken from the group consisting of trimethylammonium, amidino, guanidino, 2-amino-2-carboxyethylthio and ureido.

10. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

11. The compound of claim 9 in which is the 2-dimethylaminoethyl ester of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

12. The compound of claim 9 which is Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid.

13. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium-2-octenoic acid inner salt.

14. The compound of claim 9 which is Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium-2-octenoic acid inner salt.

15. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid.

16. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-amidino-2-octenoic acid.

17. The compound of claim 9 which is Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid.

18. The compound of claim 9 which is 6-(L-2-amino-2-carboxyethylthio)-2-(2,2,-dimethylcyclopropanecarboxamido)-2-hexenoic acid.

19. The compound of claim 9 which is 7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid.

20. The compound of claim 19 in the sodium, potassium, calcium or magnesium salt form.

21. The compound of claim 1 in which R^2 is 2,2-dihalocyclopropyl.

22. The compound as claimed in claim 1, in which R^2 is cycloalkyl of three to six carbon atoms substituted by two alkyl substituents of one to three carbon atoms each, witho the proviso that R^2 cannot contain more than ten carbon atoms.

23. A pharmaceutical composition comprising a compound as claimed in claim 1 in an amount sufficient to inhibit the activity of dipeptidase, and a pharmaceutically acceptable carrier.

24. A method of inhibiting the activity of dipeptidase in a mammal in need thereof, comprising the step of administering to said mammal a pharmacologically effective amount of the compound as claimed in claim 1.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,147,868
APPLICATION NO. : 07/839725
DATED : September 15, 1992
INVENTOR(S) : Donald W. Graham et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

IN THE RELATIONSHIP TO PRIOR APPLICATIONS, COLUMN 1, LINE 15, after "Feb. 10, 1983, now aban-doned," insert:

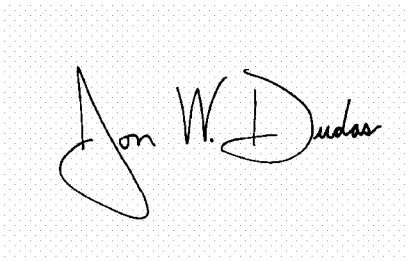
--which was a continuation of application Ser. No. 06/188,178, filed Sept. 17, 1980, now abandoned, --

IN THE TITLE PAGE, SECTION (63), LINE 7, after "Feb. 10, 1983, abandoned," insert:

--which is a continuation of Ser. No. 188,178, filed Sept. 17, 1980, abandoned,--

Signed and Sealed this

Sixth Day of November, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office

EXHIBIT 2

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

MERCK & CO., INC., Plaintiff, v. RANBAXY INC., and RANBAXY LABORATORIES LIMITED, Defendants.	)	C.A. No. ~~_____~~07-229 (GMS)

FIRST SUPPLEMENTAL COMPLAINT FOR PATENT INFRINGEMENT

Pursuant to Rule 15(d) of the Federal Rules of Civil Procedure, Plaintiff Merck & Co., Inc. ("Merck"), ~~by its undersigned attorneys, brings this action~~hereby submits this First Supplemental Complaint for Patent Infringement against defendants, Ranbaxy Inc. and Ranbaxy Laboratories Limited (collectively "Defendants")~~, for patent infringement, and~~. Merck alleges as follows:

PARTIES

1. Plaintiff Merck is a corporation incorporated under the laws of New Jersey with its principal place of business at One Merck Drive, Whitehouse Station, New Jersey 08889.

2. On information and belief, defendant Ranbaxy Inc. is a corporation organized and existing under the laws of the state of Delaware, having a principal place of business at 600 College Road East, Princeton, New Jersey 08540. On information and belief, Ranbaxy Inc. is engaged in the development, manufacturing, marketing and sale of pharmaceutical products in the United States, and conducts business in the state of Delaware.

3. On information and belief, defendant Ranbaxy Laboratories Limited ("Ranbaxy Labs") is a corporation organized and existing under the laws of India, having its principal place of business at Plot No. 90, Sector 32, Gurgaon-122 001, Haryana, India. On information and belief, Ranbaxy Labs is engaged in the development, manufacture, marketing and sale of pharmaceutical products in the United States, and conducts business in the state of Delaware. On information and belief, Ranbaxy Inc. is the wholly owned subsidiary of Ranbaxy Labs and is the agent of Ranbaxy Labs in the United States.

4. On information and belief, the acts of Ranbaxy Inc. asserted herein were done at the direction of, or with the cooperation, participation and assistance of, Ranbaxy Labs.

JURISDICTION AND VENUE

5. This is an action for patent infringement arising under the patent laws of the United States, 35 U.S.C. § 101, *et seq.*, and for declaratory judgment of patent infringement arising under 28 U.S.C. §§ 2201 and 2202 and the patent laws of the United States, 35 U.S.C. § 101, *et seq*. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a) and pursuant to 28 U.S.C. §§ 2201 and 2202.

6. Venue is proper in this Court pursuant to 28 U.S.C. §§ 1391(c) and (d) and 1400(b).

MERCK'S PATENT

7. On September 15, 1992, the United States Patent and Trademark Office ("Patent Office") duly and lawfully issued United States Patent No. 5,147,868 ("the '868 patent," a copy of which is attached as Exhibit A), entitled THIENAMYCIN RENAL PEPTIDASE INHIBITORS, to Merck as assignee of the inventors Donald W. Graham, Edward F. Rogers and Frederick M. Kahan. At all times subsequent to issuance of the '868 patent,

Merck has been the owner of the entire right, title and interest in and to the '868 patent, including the right to sue and recover for infringement. The term of the '868 patent expires September 15, 2009. The claims of the '868 patent cover, *inter alia*, the compounds cilastatin and cilastatin sodium.

8. Merck currently sells PRIMAXIN® I.M. which is an injectable suspension containing imipenem and cilastatin sodium. Merck also currently sells PRIMAXIN® I.V., which is an injection containing imipenem and cilastatin sodium.

9. Merck is the holder of approved New Drug Applications ("NDAs") under Section 505 of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 355, for imipenem and cilastatin for injectable suspension (NDA 50-630) and imipenem and cilastatin for injection (NDA 50-587).

DEFENDANTS' ACTIONS

10. ~~On information and belief,~~Prior to the filing of the original Complaint on April 30, 2007, Defendants filed an Abbreviated New Drug Application ("ANDA") for imipenem-cilastatin and associated drug master file(s), seeking approval to engage in the commercial manufacture, use, and sale of injectable products comprising imipenem and cilastatin sodium ("ANDA products") before the '868 patent expires.

11. By letter dated January 22, 2007, Defendants sent written notice of their filing to Merck, which notice was received by Merck. The notice alleged that Defendants' ANDA products will not infringe any valid claim of the '868 patent. Defendants also informed Merck that Defendants are seeking approval from the FDA to market the ANDA products before the '868 patent expires and that Defendants plan to begin marketing the ANDA products

immediately upon approval. Defendants sought a covenant from Merck not to sue under the '868 patent. Merck did not give Defendants a covenant not to sue.

12. On information and belief, Defendants are systematically attempting to meet the applicable regulatory requirements to obtain FDA approval for the ANDA products. On information and belief, Defendants have developed and tested the ANDA products. On information and belief, Ranbaxy Labs already manufactures and sells a pharmaceutical composition containing cilastatin or cilastatin sodium outside the United States. On information and belief, Defendants are preparing to import the ANDA products into the United States or manufacture the ANDA products in the United States. On information and belief, Defendants have the capacity to begin marketing and manufacturing the ANDA products immediately upon receiving regulatory approval from the FDA.

COUNT I - DECLARATORY JUDGMENT

13. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-12 as though fully set forth herein.

14. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent.

15. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that a suit by Merck will be forthcoming.

16. Defendants' manufacture, use, sale or offer for sale of the ANDA products in the United States or importation of the ANDA products into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

17. An actual controversy now exists between Merck and Defendants with respect to the infringement of the ‘868 patent.

18. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the ‘868 patent.

19. Merck does not have an adequate remedy at law.

20. Merck should be granted relief provided by 35 U.S.C. §283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants’ ANDA products will infringe the ‘868 patent and an order of this Court preliminarily and permanently enjoining them from manufacturing, using, selling and offering for sale the ANDA products.

21. On information and belief, Defendants were aware of the existence of the ‘868 patent and were aware that the marketing, manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the ‘868 patent.

22. Defendants’ written notice of the factual and legal bases for its opinion regarding the alleged invalidity and noninfringement of the ‘868 patent is devoid of an objective good faith basis in either the facts or the law.

23. On information and belief, Defendants have acted with willful disregard for Merck’s patent rights.

24. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys’ fees under 35 U.S.C. § 285.

COUNT II - PATENT INFRINGEMENT

25. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-24 as though fully set forth herein.

26. On information and belief, Defendants filed an ANDA under Section 505(j) of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 355(j), for a drug claimed in the '868 patent.

27. Defendants seek approval of their ANDA to engage in the commercial manufacture, use or sale of a drug or drug formulation claimed in the '868 patent before it expires.

28. Defendants have infringed the '868 patent under 35 U.S.C. § 271(e)(2).

29. Merck should be granted relief provided by 35 U.S.C. § 271(e)(4), including an order of this Court that the effective date of the approval of Ranbaxy's ANDA be a date that is not earlier than the present expiration date of the '868 patent, or any later expiration of exclusivity to which Merck is or becomes entitled and an order of this Court preliminarily and permanently enjoining Defendants from commercially manufacturing, using, selling and offering for sale the ANDA products.

30. On information and belief, Defendants were aware of the existence of the '868 patent and were aware that the manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the '868 patent.

31. Defendants' written notice of the factual and legal bases for its opinion regarding the alleged invalidity and noninfringement of the '868 patent is devoid of an objective good faith basis in either the facts or the law.

32. On information and belief Defendants have acted with willful disregard for Merck's patent rights and have willfully infringed the '868 patent.

33. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT III - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT OF THE '868 PATENT WITH CERTIFICATE OF CORRECTION

34. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-33 as though fully set forth herein.

35. On November 6, 2007, subsequent to the filing of this lawsuit, the Patent Office duly and lawfully issued a Certificate of Correction for the '868 patent under 35 U.S.C. § 255. A copy of the '868 patent with the Certificate of Correction is attached as Exhibit B. The Certificate of Correction resulted from a Request for Expedited Certificate to Correct the Patent Under 37 CFR 1.323, filed by Merck on May 17, 2007, to correct an error on the face of the '868 patent and an error in the first paragraph of the specification related to the recitation of U.S. Application Serial Number 06/188,178.

36. On November 13, 2007, Merck provided Defendants with a copy of the Certificate of Correction.

37. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent with Certificate of Correction, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

38. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

39. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that the ANDA products will infringe the '868 patent with Certificate of Correction.

40. Defendants' manufacture, use, sale or offer for sale of the ANDA products in the United States or importation of the ANDA products into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

41. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent with Certificate of Correction.

42. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent with Certificate of Correction.

43. Merck does not have an adequate remedy at law.

44. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' ANDA products will infringe the '868 patent with Certificate of Correction and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale the ANDA products.

45. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of the ANDA products would constitute an act of infringement of the '868 patent with Certificate of Correction.

46. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

47. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT IV - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT RELATING TO DEFENDANTS' PRODUCT II

48. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-47 as though fully set forth herein.

49. In summer, 2007, subsequent to the filing of this lawsuit, Defendants filed a second ANDA ("ANDA II") for a different injectable product, which also contains imipenem and cilastatin sodium ("Product II"). Defendants' ANDA II seeks approval to engage in the commercial manufacture, use, and sale of Product II comprising imipenem and cilastatin sodium before the '868 patent expires.

50. On September 14, 2007, Defendants informed Merck that Ranbaxy had filed their ANDA II. On October 17, 2007, Defendants produced at least a portion of their ANDA II to Merck.

51. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

52. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

53. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that Product II will infringe the '868 patent.

54. Defendants' manufacture, use, sale or offer for sale of Product II in the United States or importation of the Product II into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

55. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent by Product II.

56. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent.

57. Merck does not have an adequate remedy at law.

58. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' Product II will infringe the '868 patent and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale Product II.

59. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of Product II would constitute an act of infringement of the '868 patent

60. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

61. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT V - DECLARATORY JUDGMENT OF PATENT INFRINGEMENT RELATING TO DEFENDANTS' PRODUCT III

62. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-61 as though fully set forth herein.

63. In summer, 2007, subsequent to the filing of this lawsuit, Defendants filed a third ANDA ("ANDA III") for yet another different injectable product, which contains imipenem and cilastatin sodium ("Product III"). Defendants' ANDA III seeks approval to engage in the commercial manufacture, use, and sale of Product III comprising imipenem and cilastatin sodium before the '868 patent expires.

64. On September 14, 2007, Defendants informed Merck that Defendants had filed their ANDA III. On October 17, 2007, Defendants produced at least a portion of their ANDA III to Merck.

65. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent, including activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

66. Defendants have continued to litigate this action after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

67. The acts of Defendants indicate a refusal to change the course of their actions in the face of acts by Merck sufficient to create a reasonable apprehension that Merck would assert that Product III will infringe the '868 patent.

68. Defendants' manufacture, use, sale or offer for sale of Product III in the United States or importation of Product III into the United States will constitute patent infringement under 35 U.S.C. § 271 (a), (b) or (c).

69. An actual controversy now exists between Merck and Defendants with respect to the future infringement of the '868 patent by Product III.

70. Merck will be irreparably harmed if Defendants are not enjoined from infringing or actively inducing or contributing to infringement of the '868 patent.

71. Merck does not have an adequate remedy at law.

72. Merck should be granted relief provided by 35 U.S.C. § 283 and by 28 U.S.C. §§ 2201 and 2202, including an order of this Court declaring that Defendants' Product III will infringe the '868 patent and an order of this Court preliminarily and permanently enjoining Defendants from manufacturing, using, selling and offering for sale Product III.

73. On information and belief, Defendants were aware of the existence of the '868 patent, were aware of the existence of the Certificate of Correction to the '868 patent, and were aware that the marketing, manufacture, use, offer for sale and sale of Product III would constitute an act of infringement of the '868 patent.

74. On information and belief, Defendants have acted with willful disregard for Merck's patent rights.

75. This case is an exceptional one, and Merck should be granted an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

COUNT VI - DECLARATORY JUDGMENT THAT THE '868 PATENT WITH CERTIFICATE OF CORRECTION IS NOT INVALID

76. Merck restates and incorporates by reference the allegations of the foregoing paragraphs 1-75 as though fully set forth herein.

77. On June 21, 2007, Defendants filed the Answer and Counterclaims of Defendants Ranbaxy Inc. and Ranbaxy Laboratories Limited ("Defendants' Answer and Counterclaims.") Defendants' Answer and Counterclaims asserted, as an affirmative defense and as a counterclaim, that the '868 patent was invalid for "failure to comply with one or more of the requirements of 35 U.S.C. §§ 101, 102, 103, and/or 112."

78. On November 6, 2007, the Patent Office duly and lawfully issued a Certificate of Correction to the '868 patent under 35 U.S.C. § 255.

79. On November 13, 2007, Merck provided Defendants with a copy of the Certificate of Correction.

80. On information and belief, Defendants have made meaningful preparations for, and engaged in activities directed toward, infringing the '868 patent with Certificate of Correction, including preparations and activities that occurred after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

81. Defendants have continued to litigate this action and pursue their affirmative defense and counterclaim asserting invalidity of the '868 patent after the Certificate of Correction was issued on November 6, 2007 and after Merck provided Defendants with a copy of the Certificate of Correction on November 13, 2007.

82. The acts of Defendants indicate a refusal to change the course of their actions in spite of the issuance of the Certificate of Correction.

83. An actual controversy now exists between Merck and Defendants with respect to the alleged invalidity of the '868 patent with Certificate of Correction.

84. Merck should be granted relief including an order of this Court declaring that the '868 patent with Certificate of Correction is not invalid.

PRAYER FOR RELIEF

WHEREFORE, plaintiff Merck respectfully requests that:

a. Judgment be entered that Defendants have infringed the '868 patent by submitting the aforesaid ANDA;

b. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' ANDA products;

c. A preliminary and permanent injunction be issued, pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and 28 U.S.C. §§ 2201 and 2202, restraining and enjoining Defendants, their officers, agents, attorneys and employees, and those acting in privity or concert with them, from engaging in the manufacture, use, offer for sale, or sale within the United States, or importation into the United States, of compounds or formulations as claimed in the '868 patent, or from practicing any method as claimed in the '868 patent, or from actively inducing or contributing to infringement of the '868 patent;

d. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA be a date which is not earlier than the

present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

e. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' Product II;

f. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA II be a date which is not earlier than the present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

g. Judgment be entered declaring that Defendants will infringe the '868 patent by marketing, manufacturing, using, offering for sale or selling Defendants' Product III;

h. An order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of Defendants' ANDA III be a date which is not earlier than the present expiration date for the '868 patent, or any later expiration of exclusivity to which the '868 patent is or becomes entitled to;

i. judgment be entered declaring that the '868 patent, as originally issued and with Certificate of Correction, is not invalid;

j. ~~e.~~ Judgment be entered that Defendants acted with willful disregard for Merck's rights under the '868 patent;

k. ~~f.~~ Judgment be entered that Defendants have willfully infringed the '868 patent;

l. ~~g.~~ Judgment be entered that this case is an exceptional one and that Merck should be awarded its reasonable attorneys' fees pursuant to 35 U.S.C. § 285; and

m. ~~h.~~ Such other and further relief as the Court may deem just and proper under the circumstances.

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